Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

Official Publication of the International Association for Dental Research

Editor Emeritus—WILLIAM J. GIES

Publication Committee: PAUL E. BOYLE, J. FRANK HALL, AND HAMILTON B. G. ROBINSON.

Editor: HAMILTON B. G. ROBINSON (Ohio State University). *Business Manager:* J. FRANK HALL (Indiana University). *Circulation Manager:* PAUL E. BOYLE (University of Pennsylvania).

Assistants to the Editor: PAUL C. KITCHIN AND J. B. BROWN.

Board of Editors: THE EDITOR EMERITUS, MEMBERS OF THE PUBLICATION COMMITTEE,* ALSO ONE ELECTIVE REPRESENTATIVE OF EACH SECTION OF THE ASSOCIATION:

Ann Arbor, PHILIP JAY
Baltimore, J. BEN ROBINSON
Birmingham, J. P. LAZANSKY
Boston, R. O. GREEP
Budapest, ------ ------
Chengtu, R. G. AGNEW
Chicago, GEO. B. DENTON
Cleveland, S. W. CHASE
Columbus, P. C. KITCHIN
Denmark, ------ ------
Detroit, ------ ------
Great Britain, A. E. W. MILES
Halifax, J. S. BAGNALL
Houston, S. S. ARNIM
Indianapolis, M. K. HINE
Iowa City, GEO. S. EASTON
Johannesburg, ------ ------
Kansas City, N. A. MOORE
Louisville, D. Y. BURRILL
Materials Group, N. O. TAYLOR
Mexico, S. FASTLICHT
Minnesota, S. A. LOVESTEDT
Nebraska, R. T. IRELAND
New Orleans, F. J. WOLFE
New York, FRANCES KRASNOW
Pacific Northwest, B. O. A. THOMAS
Palestine, ------ ------
Philadelphia, S. L. ROSENTHAL
Pittsburgh, E. G. MEISEL
Prague, ------ ------
Richmond, O. W. CLOUGH
Rochester, ------ ------
San Francisco, HERMANN BECKS
St. Louis, E. F. ALLEN
South Calif., R. L. RUTHERFORD
Tennessee, ------ ------
Toronto, R. G. ELLIS
Vienna, ------ ------
Washington, D. B. SCOTT
Winnipeg, E. ROY BIER

*The Editor serves as chairman of the board, the Business Manager as secretary.

Published for THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
By The C. V. Mosby Company, St. Louis 3, Mo.

Volume 31
1952

Dent. Sch
RK
1
.J86
J4
cop. 2

COPYRIGHT, 1952, BY THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Dental
Fuxon

CONTENTS

No. 1, February, 1952

PAGE

Editor's Viewpoint ---------- 1

Tuberculosis and Periodontal Disease, With Special Reference to the Collagen Fibers. *Sigurd Ramfjord, L.D.S., M.S., Ph.D.* (University of Michigan, Ann Arbor, Mich.) ---------- 5

Studies in Periodontal Disease. *Maxwell Karshan, Benjamin Tenenbaum, Daniel E. Ziskin,* and *Roslyn Friedland* (Columbia University, New York, N. Y.) ---------- 11

Identification of a Proteolytic Enzyme of the Periodontal Pocket. *Genevieve D. Roth* and *Hugh I. Myers* (The University of Kansas City, Kansas City, Mo.) ---- 18

Inhibition of Oral Spirochetes by Antibiotic Agents In Vitro. *Robert J. Fitzgerald* and *Edward G. Hampp* (National Institute of Dental Research, Bethesda, Md.) -- 20

Phosphatase and the Oral Actinomyces. *J. J. Ennever* and *B. W. Warner* (University of Kansas City, Kansas City, Mo.) ---------- 25

Radiopacity of Oral Structures as a Basis for Selecting Optimum Kilovoltage for Intraoral Roentgenograms. *Arthur H. Wuehrmann* and *William A. Curby* (Tufts College Dental School, Boston, Mass.) ---------- 27

Dust Factors Involved in the Use of the Airdent Machine. *Myron J. Van Leeuwen, A.B., D.D.S.,* and *August T. Rassano, Jr., B.S., M.S.* (Harvard School of Dental Medicine and Harvard School of Public Health, Boston, Mass.) ---------- 33

The Bactericidal Action of Certain Antiseptics on the Oral Bacteria. *L. W. Slanetz* and *Harriet Reynolds* (University of New Hampshire, Durham, N. H.) -- 35

End-Point Study of Bactericidal Effect of Antibiotics Used in Endodontics. *Louis I. Grossman* and *Constance K. Christian* (University of Pennsylvania, Philadelphia, Pa.) ---------- 42

Mechanism of Action of Sodium Azide on the Genus Candida. *Marie S. Slawson* and *Marshall L. Snyder* (University of Oregon Dental School, Portland, Ore.) ---------- 47

The Crystal Chemistry of Carbonate Apatites and Their Relationship to the Composition of Calcified Tissues. *Duncan McConnell* (The Ohio State University, Columbus, Ohio) ---------- 53

Calcium Exchange in Enamel and Dentin as Shown by Calcium^{45}. *Elizabeth E. Underwood, M.S.,* and *Harold C. Hodge, Ph.D.* (The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.) ---------- 64

Electron Microscopy of Mature Human Enamel. *David B. Scott, Marie J. Ussing, Reidar F. Sognnaes,* and *Ralph W. G. Wyckoff* (National Institutes of Health, National Institute of Dental Research, Bethesda, Md., and Harvard School of Dental Medicine, Boston, Mass.) ---------- 74

Electron Microscopy of the Enamel of Teeth in Various Stages of Development. *Reidar F. Sognnaes, David B. Scott, Marie J. Ussing,* and *Ralph W. G. Wyckoff* (Harvard School of Dental Medicine, Boston, Mass., and the National Institutes of Health, Bethesda, Md.) ---------- 85

The Effect of Topical Applications of Sodium Fluoride on the Oral Flora of Young Adults. *Norman H. Rickles, D.D.S., M.S.,* and *Hermann Becks, M.D., D.D.S.* (University of California, San Francisco, Calif.) ---------- 94

Relation of pH to the Effectiveness of Sodium Fluoride and Stannous Fluoride in Decreasing Enamel Solubility. *Joseph C. Muhler, D.D.S.,* and *Harry G. Day, Sc.D.* (Indiana University, Bloomington, Ind.) ---------- 102

PAGE

MEASUREMENT OF DENTAL CARIES INCIDENCE IN SCHOOL CHILDREN USING A SODIUM BICARBONATE DENTIFRICE. *Robert J. Bruckner, D.D.S., M.S., Thomas J. Hill, D.D.S.*, and *Betty J. Wollpert, B.S.* (Western Reserve University, Cleveland, Ohio) 105

A MARK SENSE CARD FOR RECORDING DENTAL EXAMINATION FINDINGS. *Elliott H. Pennell* and *Samuel S. Herman* (United States Public Health Service, Washington, D. C.) 113

DEPTH TESTER FOR DENTAL CARIES LESIONS. *Charles F. Bodecker, D.D.S.* (Center Moriches, N. Y. 119

EPIDEMIOLOGIC STUDIES IN DENTAL CARIES. IV. VARIABILITY OF PROGRESSION RATES OF DENTAL CAVITIES IN THE OCCLUSAL SURFACES OF SECOND MOLAR TEETH. *Julian D. Boyd, M.D., Kenneth E. Wessels, D.D.S.*, and *Robert E. Leighton, D.D.S.* (State University of Iowa, Iowa City, Iowa) 124

STUDIES ON THE EFFECT OF METALLIC SALTS ON ACID PRODUCTION IN SALIVA. I. *J. C. Forbes* and *J. D. Smith* (Medical College of Virginia, Richmond, Va.) 129

STUDIES ON THE EFFECT OF METALLIC SALTS ON ACID PRODUCTION IN SALIVA. II. *J. C. Forbes* and *J. D. Smith* (Medical College of Virginia, Richmond, Va.) 132

THE COPPER AND COBALT LEVELS OF HUMAN SALIVA AND DENTAL CARIES ACTIVITY. *Samuel Dreizen, D.D.S., Henry A. Spies, Jr., B.S.*, and *Tom D. Spies, M.D.* (Northwestern University Studies in Nutrition at the Hillman Hospital, Birmingham, Ala.) 137

FRACTURING OF THE MOLAR TEETH IN CARIES-SUSCEPTIBLE AND CARIES-RESISTANT ALBINO RATS (RATTUS NORVEGICUS). *E. C. Nakfoor, H. R. Hunt*, and *C. A. Hoppert* (Michigan State College, East Lansing, Mich.) 143

STUDIES IN THE SYRIAN HAMSTER. II. BLOOD SUGAR: METHOD AND RESULTS. *Robert E. Sullivan, B.A.*, and *Frank G. Everett, M.S., D.M.D., M.D.* (University of Oregon Dental School, Portland, Ore.) 151

No. 2, APRIL, 1952

EDITOR'S VIEWPOINT 155

PANOGRAPHIC RADIOGRAPHY. *Robert J. Nelson, D.D.S.*, and *John W. Kumpula* (University of Washington, Seattle, Wash.) 158

HOMOLOGOUS AND HETEROLOGOUS INTRAOCULAR GROWTH OF TRANSPLANTED TOOTH GERMS. *Harold S. Fleming, D.M.D.* (Yale University School of Medicine, New Haven, Conn.) 166

PRELIMINARY STUDIES ON THE PRESENCE OF ACID PHOSPHATASE IN CERTAIN ORAL MICROORGANISMS. *Robert J. Fitzgerald* (National Institute of Dental Research, Bethesda, Md.) 189

DENTAL CARIES AND GINGIVITIS IN MALES 17 TO 20 YEARS OLD. (AT THE GREAT LAKES NAVAL TRAINING CENTER.) *Maury Massler, D.D.S., M.S., William Ludwick, D.D.S., A.B., B.Sc.*, and *Isaac Schour, D.D.S., Ph.D.* (University of Illinois, College of Dentistry, Chicago, Ill., and Great Lakes Naval Training Center, Great Lakes, Ill.) 195

RELATION OF FUNCTION TO CARIES RATE. *H. H. Neumann, W. Lefkowitz*, and *N. A. Di Salvo* (Montefiore Hospital and Columbia University, New York, N. Y.) 200

ELECTROMOTIVE FORCES AND ELECTRIC CURRENTS CAUSED BY METALLIC DENTAL FILLINGS. *William Schriever* and *Louis E. Diamond* (University of Oklahoma, Norman, Okla. and University School of Medicine, Oklahoma City, Okla.) 205

A STUDY OF THE HEALING PROCESS FOLLOWING OPERATIVE INTERFERENCE WITH THE CONTINUITY OF THE RAT MAXILLA. *William Jack Dalton, A.B., M.S., M.D., D.D.S.* (Ohio State University, Columbus, Ohio) 230

THE VASCULARITY OF THE INCISOR PULP OF THE MONKEY AND ITS ALTERATION BY TOOTH RETRACTION. *Earl O. Butcher* and *A. Cecil Taylor* (College of Dentistry and the Graduate School of Arts and Science, New York University, N. Y.) 239

MORPHOLOGIC RELATIONSHIPS BETWEEN ENAMEL AND DENTIN SURFACES OF LOWER FIRST MOLAR TEETH. *Bertram S. Kraus* (University of Arizona, Tuscon, Ariz.) 248

PAGE

THE CORRECTIVE EFFECT OF PARATHYROID HORMONE ON GENETIC ANOMALIES IN THE DENTITION AND THE TIBIA OF THE *ia* RAT. *S. N. Bhaskar, I. Schour, R. O. Greep,* and *J. P. Weinmann* (University of Illinois College of Dentistry and Harvard School of Dental Medicine, Boston, Mass.) ---------- 257

THE EFFECT OF ACTH AND CORTISONE ON THE SODIUM AND POTASSIUM LEVELS OF HUMAN SALIVA. *Samuel Dreizen, D.D.S., William Niedermeier, B.S., Ann I. Reed, B.S.,* and *Tom D. Spies, M.D.* (Northwestern University Studies in Nutrition at the Hillman Hospital, Birmingham, Ala.) ---------- 271

AMMONIA PRODUCTION AND UREASE ACTIVITY IN SALIVA. *R. M. Ballantyne, M.A., J. J. Rae, Ph.D.,* and *F. H. Lawford, Ph.D.* (Consumers Research Laboratories Limited and University of Toronto, Toronto, Canada) ---------- 281

ALKALINE PHOSPHATASE IN THE JAW BONES AND TEETH OF THE ALBINO RAT AS RELATED TO ABNORMAL STATES OF BODY GROWTH, MINERAL METABOLISM, AND DEVELOPMENT. *Anna Morse* and *Roy O. Greep* (Harvard School of Dental Medicine, Boston, Mass.) 284

SOLUBILITY OF ENAMEL ON AREAS OF KNOWN HARDNESS. *Marjorie L. Swartz* and *Ralph W. Phillips* (Indiana University School of Dentistry, Indianapolis, Ind.) -------- 293

No. 3, JUNE, 1952

EDITOR'S VIEWPOINT ---------- 301

ORAL SENSORY THRESHOLDS OF PERSONS WITH NATURAL AND ARTIFICIAL DENTITIONS. *R. S. Manly, Carl Pfaffman, Donald D. Lathrop,* and *Joan Keyser.* (Tufts Dental College, Boston, Mass., and Brown University, Providence, R. I.) ---------- 305

RELATION OF DENTAL CARIES EXPERIENCE AND GINGIVITIS TO GEOGRAPHIC LOCATION AND SIZE OF COMMUNITY IN MALES 17 TO 21 YEARS OLD (AT THE GREAT LAKES NAVAL TRAINING CENTER). *Maury Massler, D.D.S., M.S.,* and *William Ludwick, D.D.S., A.B., B.Sc.* (U.S. Naval Training Center, Great Lakes, Ill., and University of Illinois, Chicago, Ill.) ---------- 313

RELATION OF DENTAL CARIES EXPERIENCE AND GINGIVITIS TO CIGARETTE SMOKING IN MALES 17 TO 21 YEARS OLD (AT THE GREAT LAKES NAVAL TRAINING CENTER). *William Ludwick, D.D.S., A.B., B.Sc.,* and *Maury Massler, D.D.S., M.S.* (United States Naval Training Center, Great Lakes, Ill., and University of Illinois, College of Dentistry, Chicago, Ill.) ---------- 319

TESTING THE UNIFORMITY OF THE P-M-A INDEX AS A MEASUREMENT OF PERIODONTAL DISEASE. *Henry C. Sandler, D.M.D., M.P.H.* (Veterans Administration Hospital, Brooklyn, N. Y.) ---------- 323

THE EFFECT OF TOBACCO SMOKE UPON SOME BACTERIA. *H. J. Turkheim, D.M.D.* (London, England) ---------- 326

ALKALINE PPOSPHATASE ACTIVITY OF HEALING GINGIVAL WOUNDS IN THE RAT. *J. R. Ring* (Washington University School of Dentistry, St. Louis, Mo.) ---------- 329

THE DENTAL TREATMENT NEEDS OF NAVAL PERSONNEL AS DERIVED FROM 1,570 CASES. *James W. Cross, D.D.S.* (Dental Corps, U. S. Navy) ---------- 335

THE EVANSTON DENTAL CARIES STUDY. IX. THE VALUE OF ROENTGENOLOGICAL VS. CLINICAL PROCEDURES FOR THE RECOGNITION OF EARLY CARIOUS LESIONS ON THE PROXIMAL SURFACES OF THE TEETH. *J. R. Blayney, M.S., D.D.S.,* and *J. F. Greco, B.S., D.D.S.* (University of Chicago, Chicago, Ill.) ---------- 341

THE EVANSTON DENTAL CARIES STUDY. X. THE CARIES EXPERIENCE RATES OF 6, 7, AND 8-YEAR-OLD CHILDREN WITH PROGRESSIVELY INCREASING PERIODS OF EXPOSURE TO ARTIFICIALLY FLUORIDATED WATER. *I. N. Hill, D.D.S., J. R. Blayney, M.S., D.D.S.,* and *Walter Wolf, B.S.C.* (University of Chicago, Chicago, Ill.) ---------- 346

EPIDEMIOLOGIC STUDIES IN DENTAL CARIES. V. PLACEMENT OF FILLINGS AS A SOURCE OF STATISTICAL ERROR OF ESTIMATE AS TO DENTAL CARIES PROGRESSION RATES. *Julian D. Boyd, M.D., John J. Cannon, D.D.S.,* and *Robert E. Leighton, D.D.S.* (State University of Iowa, Iowa City, Iowa) ---------- 354

EFFECT OF TEMPERATURE UPON THE DEXTRINIZATION OF STARCH BY SALIVARY ENZYMES. *Naomi C. Turner, Ed.M.,* and *George E. Crowell, D.M.D.* (Forsyth Dental Infirmary for Children, Boston, Mass.) ---------- 359

PAGE

A New Technique for Oral Examination of Rodents. *Erling Johansen* (University of Rochester School of Medicine and Dentistry, Rochester, N. Y.) ---- 361

Physical Properties of Dentin. *F. A. Peyton, D.Sc., D. B. Mahler, M.S.*, and *B. Hershenov, M.S.* (School of Dentistry, University of Michigan, Ann Arbor, Mich.) 366

Crystallographic Investigation of Tricalcium Phosphate Hydrate. *Aaron S. Posner* and *Samuel R. Stephenson* (National Bureau of Standards, Washington, D. C.) ---- 371

The Staining of Nerve Fibers in Teeth. *Margaret M. Powers, B.S.* (University of Texas School of Dentistry, Houston, Texas) ---- 383

Further Experiments With Intact Teeth Placed in Various Buffer Solutions. *Jacob Yardeni* (The B. Göttlieb Dental Polyclinic, Jerusalem, Israel) ---- 393

Early Influence of Methylcholanthrene on Transplanted Tooth Germs: A Histopathogenic Study. *Harold S. Fleming, D.M.D., M.Sc.* (Yale University, New Haven, Conn.) ---- 398

The Effect of Alterations of the Facial Musculature on the Facial Skeleton and Dental Arch Form of the Rat. *Sidney L. Horowitz* and *Harry H. Shapiro* (Columbia University, New York, N. Y.) ---- 412

A Rapid Radioautographic Technique for Combined Calcified and Soft Tissues. *Howard M. Myers, D.D.S., Elizabeth Jennings, M.S.*, and *Hermann Becks, M.D., D.D.S.* (University of California, San Francisco, Calif.) ---- 416

The Comparative Effects of Penicillin, Bacitracin, Chloromycetin, Aureomycin, and Streptomycin on Experimental Dental Caries and on Certain Oral Bacteria in the Rat. *Robert M. Stephan, Robert J. Fitzgerald, F. J. McClure, M. Rachael Harris*, and *Harold Jordan* (Federal Security Agency, Bethesda, Md.) --- 421

Effects of Sonic Vibration on the Numbers of Microorganisms Cultivable From Human Saliva. *N. B. Williams* and *C. F. Eikenberg* (University of Pennsylvania, Philadelphia, Pa.) ---- 428

Developmental Disturbances of Enamel in Scurvy. *Viktors Kalnins* (Upsala, Sweden) ---- 440

No. 4, August, 1952

Editor's Viewpoint ---- 453

International Association for Dental Research. Proceedings of the Thirtieth Annual Meeting, Broadmoor Hotel, Colorado Springs, Colo., March 21, 22, and 23, 1952. Compiled by *Hamilton B. G. Robinson* (Ohio State University) ---- 455

I. President's Address. *Maynard K. Hine* (Indiana University) ---- 455

II. Index of Participants and Sequence Numerals for Corresponding Abstracts 458

III-IX. General Sessions: Abstracts 1-95 ---- 459

X. Dental Materials Group: Abstracts 96-104 ---- 496

XI. Papers Read by Title: Abstracts 105-146 ---- 499

XII. Executive Proceedings and Register of Newly Elected Officers and Members 516

The Effect of the Changing Dentition on Masticatory Function. *F. R. Shiere* and *R. S. Manly* (Tufts College Dental School, Boston, Mass.) ---- 526

Variations in Structural Morphogenesis of the Human Face and Jaws. Initial Stages of Normal and Abnormal Bone Formation (Gross) Exhibited in the Cleared Embryo and Fetus. *Samuel Rabkin* (Winter Park, Fla.) ---- 535

Thermal Injury to Normal Dog Teeth: In Vivo Measurements of Pulp Temperature Increases and Their Effect on the Pulp Tissue. *V. F. Lisanti* and *H. A. Zander* (Tufts College Dental School, Boston, Mass., and University of Minnesota School of Dentistry, Minneapolis, Minn.) ---- 548

The Effect of Single Doses of Irradiation Upon the Tissues of the Upper Rat Incisor. *H. Medak, M. Weinreb, H. Sicher, J. P. Weinmann*, and *I. Schour* (College of Medicine, University of Illinois, and Loyola University School of Dentistry, Chicago, Ill.) ---- 559

PAGE

CLEANING EFFECTIVENESS OF DENTIFRICES. *Grant Van Huysen, D.D.S.*, and *Thomas M. Boyd, A.B., D.D.S.* (Indiana University School of Dentistry, Indianapolis, Ind.) 575

ORIGIN OF ENAMEL DROPS AND CEMENTICLES IN THE TEETH OF RODENTS. *Viktors Kalnins* (Upsala, Sweden) 582

SALIVARY BACTERIA. III. THE PATHOGENICITY OF ORAL STAPHYLOCOCCI. *Arnold J. Singer, Ph.D.* (Jersey City, N. J.) 591

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. SCIENTIFIC PROCEEDINGS OF THE PACIFIC NORTHWEST SECTION. Compiled by *B. O. A. Thomas* 598

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. SCIENTIFIC PROCEEDINGS OF THE MINNESOTA SECTION. Compiled by *S. A. Lovestedt* 599

No. 5, OCTOBER, 1952

EDITOR'S VIEWPOINT 601

EFFECTS OF TOTAL BODY X-RADIATION ON SALIVARY COMPONENTS OF DOGS. *J. L. Nemes, M. G. Wheatcroft*, and *R. S. Leopold* (University of Maryland and National Naval Medical Center, Bethesda, Md.) 603

THE EFFECT OF LOCAL X-RAY IRRADIATION UPON THE TEETH AND SURROUNDING TISSUES IN YOUNG AXOLOTLS (SIREDON MEXICANUM). *V. V. Brunst, E. A. Sheremetieva-Brunst*, and *Frank H. J. Figge* (University of Maryland, School of Medicine, Baltimore, Md.) 609

X-RAY CRYSTALLOGRAPHIC EXAMINATION OF CALCULI FROM SALIVARY GLANDS. *Aksel Tovborg Jensen* and *Marianne Danø* (Royal Veterinary and Agricultural College, Copenhagen, Denmark) 620

AN ARTIFICIAL MOUTH FOR CARIES RESEARCH. *Ward Pigman, Howard C. Elliott, Jr.*, and *R. O. Laffre* (University of Alabama, Birmingham, Ala.) 627

MANGANESE ANTAGONISTS IN THE NUTRITION OF LACTOBACILLUS ACIDOPHILUS. *Warner W. Carlson, Louis V. Farina*, and *Virginia Whiteside-Carlson* (University of Alabama, Birmingham, Ala.) 634

THE INFLUENCE OF THE ENAMEL ORGAN UPON THE CALCIFICATION OF DENTIN AND THE FUNCTIONS OF THE ODONTOBLASTS. *J. T. Irving* (University of Cape Town, Cape Town, South Africa) 639

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. BIOGRAPHICAL DIRECTORY OF MEMBERS, SEPTEMBER, 1952. Compiled by *Harold C. Hodge* (University of Rochester) 646

No. 6, DECEMBER, 1952

EDITOR'S VIEWPOINT 739

A TRACER STUDY OF THE TRANSFER OF F^{18} TO TEETH BY TOPICAL APPLICATION. *Howard M. Myers, D.D.S., Joseph G. Hamilton, M.D.*, and *Hermann Becks, M.D., D.D.S.* (University of California, San Francisco and Berkeley, Calif.) 743

THE EFFECT OF SODIUM FLUORIDE UPON THE SURFACE STRUCTURE OF POWDERED DENTAL ENAMEL. *Robert B. Fischer, Ph.D.*, and *Joseph Charles Muhler, D.D.S., Ph.D.* (Indiana University, Bloomington, Ind.) 751

RELATIONSHIP BETWEEN pH, AGE, AND CONCENTRATION OF SOLUTIONS OF STANNOUS FLUORIDE AND SODIUM FLUORIDE IN DECREASING ENAMEL SOLUBILITY AND AFFECTING THE UPTAKE OF FLUORINE. *Joseph C. Muhler, Harry G. Day*, and *William H. Nebergall* (Indiana University, Bloomington, Ind.) 756

THE FORMATION OF HYDROXYFLUOR-APATITE IN TOOTH ENAMEL UNDER INFLUENCE OF DRINKING WATER CONTAINING FLUORIDE. *Werner Rathje* (Max Planck-Instit für Biochemie, Tübingen, Germany) 761

THE EFFECTS OF UREA AND SALTS OF AMMONIUM PHOSPHATE ON SALIVARY AMYLASE. *Leon H. Schneyer* (Montefiore Hospital, New York, N. Y.) 767

PAGE

THE EFFECTIVENESS OF AN AMMONIUM ION TOOTH POWDER IN THE CONTROL OF DENTAL CARIES. II. COMPOSITION OF DENTIFRICES USED IN THE EXPERIMENT. *G. N. Davies* and *R. M. King* (University of Otago, Dunedin, New Zealand) ---------- 775

THE ACCESSIBILITY OF THE ORGANIC DENTINAL MATRIX. *George W. Burnett, D.D.S., Ph.D.*, and *Henry W. Scherp, Ph.D.* (University of Rochester, Rochester, N. Y.) -- 776

DENTINAL PROTEIN: AMINO ACID COMPOSITION. *W. C. Hess, Ph.D., C. Lee, M.S.*, and *B. A. Neidig, M.S.* (Georgetown University, Washington, D. C.) ---------- 791

THE ISOLATION OF CHONDROITIN SULFURIC ACID FROM DENTIN. *W. C. Hess* and *C. Lee.* (Georgetown University School of Medicine and Dentistry, Washington, D. C.) -- 793

EXPERIMENTAL DENTAL CARIES. I. THE EFFECT OF ORCHIECTOMY AND OVARIECTOMY ON DENTAL CARIES IN IMMATURE RATS. *Joseph C. Muhler, D.D.S., Ph.D.*, and *William G. Shafer, D.D.S., M.S.* (Indiana University, School of Dentistry, Bloomington and Indianapolis, Ind.) ---------- 798

THE EFFECT OF VITAMIN E DEFICIENCY ON THE RAT INCISOR. *J. J. Pindborg* (University of Illinois and the Royal Dental College, Denmark, Copenhagen) ---------- 805

THE REPARATIVE EFFECT OF ANTABUSE (TETRAETHYLTHIURAM DISULFIDE) UPON DENTAL CHANGES IN VITAMIN E DEFICIENT RATS. *J. J. Pindborg* (University of Illinois and the Royal Dental College, Denmark, Copenhagen) ---------- 812

SYMPTOMS OF VITAMIN A AND E DEFICIENCIES IN THE INCISOR OF THE RAT FED SODIUM SULFITE. *J. T. Irving, J. J. Pindborg, O. G. Fitzhugh, J. P. Weinmann*, and *I. Schour* (University of Illinois College of Dentistry, Chicago, Ill. and Federal Security Agency, Washington, D. C.) ---------- 815

FURTHER STUDIES OF A DIFFERENTIAL CULTURE TECHNIQUE FOR ESTIMATIONS OF ACIDOGENIC BACTERIA IN SALIVA. I. COMPARISON WITH CONVENTIONAL TECHNIQUES AND CONCURRENT CLINICAL EXAMINATIONS. *Dorothy R. Rizzo* and *Evelyn B. Tilden* (Northwestern University Dental School, Chicago, Ill.) ---------- 825

FURTHER STUDIES OF A DIFFERENTIAL CULTURE TECHNIQUE FOR ESTIMATIONS OF ACIDOGENIC BACTERIA IN SALIVA. II. SPECIES OF LACTOBACILLI ISOLATED FROM SALIVA AND THEIR DISTRIBUTION IN A GROUP OF CHILDREN. *Evelyn B. Tilden* and *Muriel Svec* (Northwestern University, Chicago, Ill.) ---------- 831

DENTAL CARIES EXPERIENCE IN PRIMARY TEETH. *John T. Fulton* (Children's Bureau, Washington, D. C.) ---------- 839

DENTAL CARIES EXPERIENCE OF SOME FRESHMAN COLLEGE STUDENTS IN UTAH. *Ethelwyn B. Wilcox, Ph.D., Delbert A. Greenwood, Ph.D.*, and *Leora S. Galloway, B.S.* (Utah State Agricultural College, Logan, Utah) ---------- 844

DENTAL CARIES EXPERIENCE OF A GROUP OF SCHOOL CHILDREN AT OGDEN, UTAH, WITH AND WITHOUT RHEUMATIC FEVER. *Ethelwyn B. Wilcox, Ph.D., Delbert A. Greenwood, Ph.D.*, and *Leora S. Galloway, B.S.* (Utah State Agricultural College, Logan, Utah) ---------- 849

EXCITABILITY IN TERMS OF TIME OF THE DENTAL NERVOUS ELEMENTS. *W. R. Loewenstein* and *A. Faivovich* (University of Chile, Santiago, Chile) ---------- 854

FIRST PRINCIPLES OF FOUNDATIONAL CAPACITY IN DENTAL INVESTING TISSUES. *Edwin S. Smyd* (Detroit, Mich.) ---------- 860

MUSICAL THERAPEUTICS. *Harold G. Green, D.D.S.* (Jacksonville, Fla.) ---------- 871

THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. CHICAGO SECTION. Compiled by *G. B. Denton* ---------- 875

INDEX TO VOLUME 31 ---------- 877

GENERAL MATTER FOR VOLUME 31:

TITLE PAGE ---------- i

CONTENTS OF VOLUME ---------- iii

Vol. 31 No. 1

U
O
MAR 11 1952
DENTISTRY
LIBRARY

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Editor

HAMILTON B. G. ROBINSON

FEBRUARY, 1952

Editor's Viewpoint ---- 1

Tuberculosis and Periodontal Disease, With Special Reference to the Collagen Fibers. Sigurd Ramfjord, L.D.S., M.S., Ph.D. (University of Michigan, Ann Arbor, Mich.) ---- 5

Studies in Periodontal Disease. Maxwell Karshan, Benjamin Tenenbaum, Daniel E. Ziskin, and Roslyn Friedland. (Columbia University, New York, N. Y.) ---- 11

Identification of a Proteolytic Enzyme of the Periodontal Pocket. Genevieve D. Roth and Hugh I. Myers. (The University of Kansas City, Kansas City, Mo.) ---- 18

Inhibition of Oral Spirochetes by Antibiotic Agents In Vitro. Robert J. Fitzgerald and Edward G. Hampp. (National Institute of Dental Research, Bethesda, Md.) ---- 20

Phosphatase and the Oral Actinomyces. J. J. Ennever and B. W. Warner. (University of Kansas City, Kansas City, Mo.) ---- 25

Radiopacity of Oral Structures as a Basis for Selecting Optimum Kilovoltage for Intraoral Roentgenograms. Arthur H. Wuehrmann and William A. Curby. (Tufts College Dental School, Boston, Mass.) ---- 27

Dust Factors Involved in the Use of the Airdent Machine. Myron J. Van Leeuwen, A.B., D.D.S., and August T. Rossano, Jr., B.S., M.S. (Harvard School of Dental Medicine and Harvard School of Public Health, Boston, Mass.) ---- 33

(*Contents continued on inside back cover*)

Published for THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
By The C. V. Mosby Company, St. Louis 3, Mo.

Copyright 1952 by The International Association for Dental Research

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Editor Emeritus—William J. Gies

Publication Committee—J. Frank Hall, Paul E. Boyle, and Hamilton B. G. Robinson.

Editor—Hamilton B. G. Robinson (Ohio State University). *Business Manager*—J. Frank Hall (Indiana University). *Circulation Manager*—Paul E. Boyle (University of Pennsylvania).

Assistants to the Editor—Paul C. Kitchin and J. B. Brown.

Board of Editors—Editor emeritus, members of the Publication Committee, and sectional representatives named in the fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1951)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
North America	Halifax	J. S. Bagnall	J. S. Bagnall	J. S. Bagnall
	Toronto	A. W. Ellis	A. W. Ellis	A. W. Ellis
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
	Ann Arbor	Philip Jay	Philip Jay	Philip Jay
	Baltimore	M. S. Aisenberg	E. C. Dobbs	J. Ben Robinson
	Birmingham	L. Robinson	J. F. Volker	J. P. Lazansky
	Boston	P. H. Keyes	R. S. Manly	R. O. Greep
	Chicago	E. B. Tilden	G. R. Lundquist	G. B. Denton
	Cleveland	S. W. Chase	T. J. Hill	S. W. Chase
	Columbus	H. B. G. Robinson	L. F. Edwards	P. C. Kitchin
	Detroit		P. C. Lowery	
	Houston	I. R. Telford		S. S. Arnim
	Indianapolis	R. W. Phillips	J. F. Hall	M. K. Hine
	Iowa City	A. O. Klaffenbach	A. W. Bryan	G. S. Easton
	Kansas City	Ben W. Warner	H. L. Myers	N. A. Moore
	Louisville	R. E. Myers	D. Y. Burrill	D. Y. Burrill
	Minnesota	D. F. Chernausek	W. D. Armstrong	S. A. Lovestedt
	Nebraska	D. A. Keys	D. A. Keys	R. T. Ireland
	New Orleans	H. W. Peterson	H. W. Peterson	F. W. Wolfe, Sr.
	New York	Isaac Neuwrith	G. Bevelander	F. Krasnow
	Pacific Northwest	N. M. Phatak	H. J. Noyes	B. O. A. Thomas
	Philadelphia	N. B. Williams	P. E. Boyle	S. L. Rosenthal
	Pittsburgh	T. W. Brand	W. F. Swanson	E. G. Meisel
	Richmond	H. D. Coy	A. D. Brashear	O. W. Clough
	Rochester	J. W. Hein	E. R. Costich	
	San Francisco	S. Epstein	W. C. Fleming	Hermann Becks
	St. Louis	E. A. Jasper	W. B. Gurley	E. F. Allen
	Southern Calif.	L. A. Bavetta	B. Z. Rabinowitch	R. L. Rutherford
	Tennessee	J. T. Ginn	O. A. Oliver	
	Washington	J. S. Oartel	F. A. Arnold, Jr.	D. B. Scott
Not Belonging to a Division	Budapest	Ervin Lörinczy		
	Chengtu	R. G. Agnew	R. G. Agnew	R. G. Agnew
	Denmark	P. O. Pederson		
	Johannesburg	J. Staz		
	London	M. A. Rushton		
	Mexico	F. R. Leycegui	F. R. Leycegui	S. Fastlicht
	Palestine	S. Lewin-Epstein		
	Prague	F. Neumann		
	Austria, Vienna Center	F. Driak		
Dental Materials Group		R. W. Phillips	F. A. Peyton	N. O. Taylor

OFFICERS OF THE ASSOCIATION

President—L. S. Fosdick, *President-elect*—M. K. Hine, *Vice-president*—F. A. Arnold, Jr., *Secretary-Treasurer*—E. H. Hatton, *Assistant to the Secretary-Treasurer*—D. Y. Burrill, *Editor*—H. B. G. Robinson, *Trustees*—H. Trendley Dean, P. C. Kitchin, T. J. Hill, H. C. Hodge, L. M. Waugh.

Vol. 31, No. 1, February, 1952. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription price: United States, its Possessions, Pan-American Countries, $9.00 per volume; Canada and other foreign countries, $9.50. Single number when available, $1.25. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, W. J. Gies, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): . . . "(a) To promote broadly the advancement of active research in all branches of dental science and in related phases of the sciences that contribute directly to the development of dentistry, and which add to the knowledge of the mouth and teeth and of their relations to the body as a whole; . . . (b) to encourage and facilitate coöperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology; (c) to the end, particularly, that dentistry may render cumulatively more perfect service to humanity." (The Association has been using "dentistry" and "stomatology" as synonyms.)

Membership: total (Nov. 10, 1946), 627. *Eligibility (Const., Art. 14, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to stomatology, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of June 1, 1949): $1,506.06. There have been no expenditures from this fund.

Notices

The 30th General Meeting of the International Association for Dental Research will be held at the Broadmoor, Colorado Springs, March 21, 22, and 23, 1952.

Subsection Nd-Dentistry—of the A.A.A.S. will meet in Philadelphia on December 28 and 29. A symposium on Fluoridation as a Public Health Measure will be presented.

INFORMATION FOR PROSPECTIVE CONTRIBUTORS, SUBSCRIBERS, OR ADVERTISERS

The JOURNAL OF DENTAL RESEARCH was dedicated by its founder to the dissemination of knowledge pertaining to the teeth and mouth, and to their relations to the body as a whole. It publishes original investigations in these and related fields. Articles are accepted only on condition they are contributed solely to this JOURNAL. Articles appearing in other domestic or foreign periodicals will not be reprinted in the JOURNAL except in extraordinary cases. The JOURNAL will reproduce a limited number of half-tones or zinc etchings without cost to the author. References to the literature must conform to the following style:

1. (To a periodical)—Doe, J. A.: Dental Disease, *J. D. Res.* **16**: 42, 1937.
2. (To a book)—Doe, J. A.: The Dental Practice, ed. 2, Chicago, 1930, Smith, ch. 2, p. 114.

See McBride, *J. D. Res.* **16**: 537, 1937.

Titles should be given for all references. Papers presented at society meetings should be so footnoted. Reprints, up to 500 copies, will be supplied at prices quoted by the publisher after the JOURNAL is in the mail, and must be ordered from the publisher. Additional reprints may be purchased only at the discretion of the Board of Editors.

Subscription price for the volume (issued bimonthly beginning with February) is $9.00; Foreign, $9.50. Single copies $1.85. The price of volumes and single numbers prior to 1948 will be quoted upon request to the office of the Business Manager, 1121 W. Michigan St., Indianapolis, Ind.

Matters pertaining to subscriptions, change of address, and other business matters should be addressed to The C. V. Mosby Company, Publishers, 3207 Washington Blvd., St. Louis 3, Mo.

Address all communications relating to editorial matters to the JOURNAL OF DENTAL RESEARCH, Ohio State University, College of Dentistry, Columbus 10, Ohio; advertising or business matters to the JOURNAL OF DENTAL RESEARCH, The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo.

EDITOR'S VIEWPOINT

Another of the theories that has been repeated often enough as to be accepted as fact is delivered a fatal blow by careful study. Ramfjord (p. 5) was unable to demonstrate any clinical, radiographic, or histological changes in the periodontal tissues that could be attributed to tuberculosis in a group of tuberculous monkeys. He gives as his opinion that periodontal disease observed in tuberculous patients is due to poor oral hygiene, not to systemic changes caused by tuberculosis.

Karshan, Tenenbaum, Ziskin, and Friedland (p. 11) have cleared away more of the mythical relationship claimed to exist between periodontal disease and systemic disease. Their thorough study indicates that while systemic diseases may contribute to periodontal diseases, there is no consistent pattern and there is no indication that a particular systemic factor, measurable by usual clinical or laboratory methods, exists as a constant. As the authors suggest, some general changes, i.e., leukocytosis, erythrocyte sedimentation rate and serum phosphate may be the result of periodontal disease rather than its cause.

Although Roth and Myers' report (p. 18) may appear limited and simple, their demonstration of collagenase in periodontal pockets gives statements based on conjecture more tangible basis. Further investigations may show the relative importance of various necrotizing enzymes.

A study by Fitzgerald and Hampp (p. 20) shows that a number of antibiotics are effective at low concentrations against oral spirochetes. This indicates that these drugs might well replace agents which are more destructive to oral tissues in this phase of treatment of necrotizing ulcerative gingivitis. However, it should be pointed out that most clinicians appear to regard drug treatment only as a minor part of the therapy for this type of gingival disease.

Ennever and Warner (p. 25) show phosphatase activity of the oral actinomycetes which are inhabitants of dentobacterial plaques, and probable "binders" of calculus. This is of significance in confirming some earlier hypotheses of the possible participation of these filamentous forms in calculus formation.

Wuehrmann and Curby (p. 27) show that the optimal kilovoltage for intraoral radiography appears to be 90 to 95 kvp. They used experimental methods on ivory to develop their study and used ivory step plates intraorally to evaluate the various kilovoltage levels. This report is an example of the opportunity for valuable research in the field of clinical dentistry as well as in the areas of biological investigation.

From studies of the concentration of dust particles in a room where an Airdent machine was in operation, Van Leeuwen and Rossano (p. 33) report that the machine and technique do not appear to present a health hazard to the patient or operator. Other investigations on the effect on the tissues or the abrasive used in the airbrasive technique are in progress. The chief alarm seems to arise from those who fail to differentiate nontoxic dusts from toxic dusts. As the authors point out, aluminum oxide is not a toxic dust unlike the familiar toxic dust, silica.

Slanetz and Reynolds (p. 35) found the organisms of the normal oral flora greatly reduced in number for as long as 5 hours after use of antiseptic or antibiotic solutions and troches and found appreciable reduction even five days later. The value of supressing, but not eliminating, the oral organisms, is not evident. If certain harmful groups, as the acidogens, might be eliminated or reduced to negligible numbers by use of a troche or mouthwash every fifth day, the value is more obvious. The importance of reduction of the total flora from 159 millions to 5 millions is questionable.

Grossman and Christian (p. 42) have determined the lowest concentration at which penicillin, bacitracin, streptomycin, and caprylate sodium are effective (in vitro) against representative organisms. These organisms were selected on the basis of observations of 1017 root canal cultures. No one of the agents tested was effective against all of the common root canal pathogens. It appears that at the present time a combination of agents offers the best approach to the root canal therapy.

Slawson and Snyder (p. 47) confirm Diamond and Walstad's observation (*J. D. Res.* **29**: 8, 1950) that sodium azide inhibits the growth of yeasts and molds on tomato juice agar used for oral lactobacillus counts. They found the effect to be the result of the action of sodium azide on the iron porphyrin pigments.

McConnell (p. 53) discusses the crystal chemistry of francolite in relationship to enamel. He represents a modification of his and Gruner's earlier theory and hypothesizes a crystalline structure for francolite. On this basis, he considers the probable crystalline structure of enamel. This subject is far from closed and increased knowledge of the submicroscopic structure of enamel may contribute to better methods of increasing enamel resistance.

Although the crystalline structure of enamel and dentin may be similar or identical, there are many chemical and physical differences between the two tissues. Underwood and Hodge (p. 64) have demonstrated one of these differences by studies of adsorption of calcium.[45] Again, the importance of making separate studies of enamel and dentin, rather than using whole teeth, is emphasized.

Scott, Ussing, Sognnaes, and Wyckoff (p. 74) used the electronic microscope to study sections from mature enamel. They found most interesting patterns in the organic framework. A sub-microscope fibrillar network has been demonstrated. The limitations imposed by optical microscopes have been overcome by this technique as well as by crystalline studies using polarized light and x-ray diffraction. Further detailed observation coupled with careful interpretation may give us important knowledge far beyond that conceived by microscopists of a few decades ago. Beust, Kitchin, and others envisioned much of this but were limited by available equipment.

Sognnaes, Scott, Ussing, and Wyckoff (p. 85) have observed, with the electron microscope, the enamel of rats and monkeys in varying stages of development. The fibrillar nature of mature enamel appears to develop from a homogeneous organic structure. Crystal-like particles have been observed but not identified.

The method by which fluorides inhibit caries has never been adequately explained. It is felt by many that the reduction in new caries exhibited after topical application of fluorides is not due to decreased solubility of enamel, alone. One theory has been that fluorides act as enzyme inhibitors interfering with degradation of carbohydrates by bacteria. Rickles and Becks (p. 94) tested this hypothesis but found that topical applications of fluorides do not alter acidogenic properties of the oral flora, nor the salivary lactobacillus index. While these findings do not support the enzyme inhibitor theory, they do not disprove it. Their figure 1 should be carefully studied as it appears to show that topically applied fluorides do alter the production of acid by oral bacteria, but statistical analysis proves this an illusion. Less striking data have been used to support theories without using the scientific approach utilized by Rickles and Becks.

It is unfortunate that acidulated fluorides were recommended for topical application and used widely on the basis of laboratory studies. Later chemical studies showed the inefficiency of these fluoride solutions. Now, Muhler

and Day (p. 102) report new laboratory procedures showing the errors of the earlier report. Had the earlier studies recognized these errors much useless therapy might have been avoided.

Bruckner, Hill, and Wollpert (p. 105) found sodium bicarbonate dentifrice to be of no more significant value in controlling caries than a dentifrice without sodium bicarbonate under conditions of study. It is questionable to us whether studies of the possible effectiveness of dentifrices should be studied by their use at one time during the day. It is true that comparisons of medicated and unmedicated dentifrices are well "controlled" under those conditions, i.e., the only variant is the therapeutic agent, but such studies do not make the comparisons under most optimal conditions or under the most natural conditions. To test the former the comparison should be made between dentifrices used immediately after meals. To study the effectiveness under usual conditions of use the two dentifrices should simply be distributed to the two groups for home use under ordinary methods of home care. Bruckner, Hill, and Wollpert also report comparisons of different methods of caries activity evaluation.

Pennell and Herman (p. 113) report on the use of a "sense" card to record findings of dental examinations. It includes data on DMF teeth, extraoral and intraoral pathosis, periodontal disease, enamel dystrophies and occlusion. The results of examination of the Boy Scouts at the Valley Forge Jamboree are compared with the data from Hagerstown. It is noted that the examination-recording time averaged less than 2 minutes per individual. We cannot conceive of a 2 minute examination being adequate for caries observation, alone, even without the other portions of the examination, no matter how efficient the recording technique may be. At best, such examinations are only the crudest of screening operations. While it may be argued that the errors will cancel each other in examinations of this type, it cannot be denied that they are wholly inadequate and we personally question the validity of comparisons based on data derived in such a manner. The recording technique should be valuable when more careful and thorough examinations are utilized.

The problem of cavity depth in relationship to the progress of caries has stimulated Bodecker (p. 119) to devise an instrument to reduce subjectivity in examination of caries at different times. It would be most interesting to secure values on a group such as studied by Boyd, Wessels, and Leighton (p. 124), using the instrument devised by Bodecker. It must be pointed out that the measurement of cavity progression is not a valid method of evaluating caries preventive measures directed at stopping the initiation of caries. For example, proponents of the topical fluoride method of caries control point out that this technique does not halt progression of cavities already initiated but only prevents the beginning of a percentage of new cavities.

Boyd, Wessels, and Leighton (p. 124) report the rate of progress of caries in second molars of a group of children from a school for the feeble minded. Such data are interesting for few groups of children with completely neglected cavities are observable over a period of years. The authors found the progress of carious lesions relatively slow after the initiation of the cavity. They recommend that caries therapy should be tested in mouths where no cavities are placed, contending that the placement of restorations is in itself anticaries therapy. However, the caries control methods now being recommended are aimed at controlling the process of dental caries and thereby preventing the initiation of cavities. In such instances, the progress of cavities after their initiation is unimportant from the standpoint of evaluation of the technique.

The search for antibacterial agents that will inhibit caries when used in dentifrice or mouthwash continues. Forbes and Smith (p. 129 and p. 132) have investigated the effect of metallic salts in reducing acid production in salvia. They found several metallic salts effective and tried copper and nickel salts in human subjects. Copper and nickel salts both inhibited oral acid production but copper salts had a metallic taste and left a discoloration. These objectional properties would prevent the general use of the agents but should not prevent further studies.

Dreizen, Spies, and Spies (p. 137) found traces of copper and cobalt in saliva but no nickel or molybdenum. Copper was present at levels of 10 to 47.5 mg. per 100 c.c. and cobalt as high as 12.5 mg. per 100 c.c., but at these levels neither element influences acid production nor the growth of an oral strain of lactobacilli Forbes and Smith (p. 129) have demonstrated that copper salts added to the oral cavity may inhibit acid production. These studies suggest that copper is not a natural salivary factor acting against dental caries. Further studies of this type may uncover some factor which acts, under normal conditions, to inhibit the caries process.

Nakfoor, Hunt, and Hoppert (p. 143) found that the molars of genetically caries-resistant rats were more resistant to fracturing than those of caries-susceptible live rats. They gained the impression that the crevices of the rat molar in caries-resistant lines are narrower than the crevices in susceptible lines. In spite of the observation that the teeth of susceptible animals fracture more readily when struck on the cusps they conclude that fracturing is not an important factor in initiating caries in their rats. The fracture may be produced by food particles impacted between cusps and in this instance the width of the cusp and the reduced fracture resistance may be key factors in the caries resistance difference.

Sullivan and Everett (p. 151) describe a method for obtaining blood from the hamster. Although they used this technique to obtain blood for sugar level studies, it is applicable for other uses. The increasing use of the hamster as a laboratory animal requires more knowledge of its physiologic chemistry and anatomy as well as of its pathology.

TUBERCULOSIS AND PERIODONTAL DISEASE, WITH SPECIAL REFERENCE TO THE COLLAGEN FIBERS

SIGURD RAMFJORD, L.D.S., M.S., PH.D.

From the University of Michigan School of Dentistry, Department of Oral Pathology and Periodontia, Ann Arbor, Mich.

A NUMBER of chronic debilitating diseases, including tuberculosis, have been considered predisposing or contributing factors in the development of periodontal disease.[2, 6, 10, 31] Some authors[28, 33, 37] have even claimed that the periodontal lesions associated with these systemic diseases are characteristic enough to be helpful in arriving at a diagnosis of the systemic disease. A mild, painless, specific type of gingivitis associated with pulmonary tuberculosis was described by Cahn,[8] who also reported "roentgenographic evidence of enlarged, swollen intertrabecular spaces between and around the teeth." Cipes[9] examined more than one thousand tuberculous patients and found a high incidence of characteristic gingivitis which varied in severity and extent proportionately to the pulmonary involvement. A definite association between pulmonary tuberculosis and periodontal lesions was also reported by Kramer[21] and Brody.[5] They also reported periodontal bone changes, and were later supported by Goadby,[14] who attributed these bone changes in the jaw to a loss of lime salts and a subsequent formation of granulation tissue. A general withdrawal of calcium salts from the bony system associated with pulmonary tuberculosis might, according to Miller,[24] cause a horizontal wasting of the alveolar process. This theory of demineralization of bone in cases of pulmonary tuberculosis seems to be unfounded.[36]

A second group of investigators have found, in tuberculous patients, an increased occurrence and extent of the commonly recognized types of periodontal diseases.[13, 32] These reports include a large number of clinical cases and a significant relationship is claimed between pulmonary tuberculosis and periodontal disease, utilizing healthy individuals as controls. In several textbooks of oral pathology[1, 7, 20] general statements are made about pulmonary tuberculosis causing lowered systemic and local resistance to irritation and infection, and thereby predisposing to periodontal disease.

The third group of reports is headed by Tanchester and Sorrin's thorough investigation of 1,500 tuberculous patients plus a large number of healthy controls. They did not find any pathognomonic dental or periodontal signs of tuberculosis, nor did they observe more dental or periodontal disease in tuberculous than in healthy individuals. Roentgenographic examination of the teeth and their supporting structures did not reveal any changes which could be attributed to tuberculosis. Similar statements have been made by Brodsky,[4] Ordene,[27] and Gruber[18] after examination of a large number of tuberculous patients.

Received for publication, June 1, 1951.
From a thesis submitted for the degree of Ph.D. at the University of Michigan.

Most of the reports are based on clinical impressions rather than on scientifically controlled investigations, making an evaluation of the literature impossible. The etiology of periodontal disease is considered by many investigators to be obscure, local factors are often difficult to observe, and systemic disease, when present, offers a seemingly plausible explanation of the periodontal involvement. This "systemic factor escape" in the diagnosis of periodontal disease is a vague concept which has been indiscriminately used and which has impaired the progress in dentistry tremendously.

INVESTIGATION

Clinical examination followed by necropsy and histologic study was performed in 10 rhesus monkeys which suffered from severe tuberculosis of the human type. Seven healthy monkeys of approximately the same age which lived under the same environmental conditions were used as controls. The tuberculous animals were sacrificed when they reached the moribund stage of their disease. Clinical oral and grossly visible visceral changes were recorded, oral photographs and dental roentgenograms were taken, and models were made of their teeth. Specimens for microscopic study were taken from jaws, teeth, temporomandibular joints, mesial end of one tibia, and from visceral organs, including the lung, peribronchial and mesenteric lymph nodes, stomach, liver, kidney, spleen, adrenals, and pancreas. Hematoxylin and eosin stained sections were utilized for examination of all of the tissues. The seven control animals were sacrificed and examined in the same way as the experimental animals.

A summary of the case reports is given in Table I. Nine of the monkeys had, at the time of autopsy, extensive miliary spread of pulmonary tuberculosis with lesions in spleen, liver, kidney, pancreas, and peribronchial and mesenteric lymph nodes. The tenth monkey had severe renal tuberculosis. They all showed evidence of catarrhal gastritis and colitis, and a few animal parasites were found in 3 of the monkeys. Four of the experimental animals had clinically normal gingiva, while the other 6 showed varying degrees of materia alba, dental calculus, and a mild marginal gingivitis of the same type as seen in the control animals. The gingivitis was seen only in association with local irritants. The dental roentgenograms did not reveal any bone changes and microscopically no difference could be observed between the periodontal tissues in the experimental and the control animals. Various manifestations of traumatism, in some instances histologically resembling diffuse atrophy or periodontosis,[26] were present in both groups. The implications of these findings have been discussed in a previous article.[29]

Since degeneration of periodontal fibers has been listed as the initial and most important finding in periodontosis,[3, 15, 17] it was felt that a logical approach to the problem of possible periodontal impairment in systemic disease (here tuberculosis) would be a thorough investigation of the collagen for general changes, for example in joint capsules and for changes localized in the periodontal tissues. The collagenous fibers are a characteristic constituent of

all types of connective tissue, and there is no more important group of substances in dental physiology and pathology than the collagen group of fibroproteins.[11]

In the loose connective tissue the collagenous fibers are long, straight or wavy, colorless threads or ribbons which run in all directions.[23] In connective tissue, which performs a definite function, they are arranged parallel in such a way as to allow the function to be transferred as a pull to the collagenous fibers. They consist of extremely fine, uniform, parallel, collagenous fibrils which are held together by a cement substance. The fibers are very flexible, but not elastic, and offer a great resistance to a pulling force.

A satisfactory definition of collagen has not been devised. Morphologists have gained a common concept of the meaning of the term through descriptive terminology and regard collagen as a homogenous, fibrillary, intercellular protein substance which can be distinguished from other intercellular materials by its staining reactions, microscopic structure, and other empirical properties. The source of collagen has not been proved definitely. It appears in the

TABLE I

MONKEYS WITH TUBERCULOSIS*

MONKEY NUMBER	AGE YEARS	CLINICAL PERIODONTAL DIAGNOSIS	MATERIA ALBA AND/OR CALCULUS	MICROSCOPIC PERIODONTAL DIAGNOSIS
1514	3½	Mild gingivitis Stillman's cleft	+	Mild chronic gingivitis
1775	3	Normal gingiva	–	Normal gingiva
1641	4	Normal gingiva	–	Mild chronic gingivitis
1643	3½	Mild gingivitis	+	Mild chronic gingivitis
1950	2½	Mild gingivitis	+	Chronic gingivitis
1328	4-5	Normal gingiva	–	Mild chronic gingivitis
1921	2	Normal gingiva	–	Mild chronic gingivitis
1842	2	Mild gingivitis	+	Gingivitis
I	2	Gingivitis	+	Chronic gingivitis
1771	5-6	Mild gingivitis	+	Mild chronic gingivitis

*The dental roentgenograms were negative. All of the seven control animals showed signs of gingivitis, both clinically and microscopically.

intercellular medium only in the presence of fibroblasts, and Hass and McDonald[19] showed in tissue cultures that new deposits of collagen appeared only in the immediate vicinity of a new growth of fibroblasts.[38] It was also found that new collagen did not appear in increasing quantities or persist for a significant interval of time around fibroblasts which showed morphological evidence of diminished viability. The first detectable stage in the process of resorption of collagen was a loss of the selective staining property. One could assume that the same phenomena would initiate periodontosis. A depression of the pH level of the medium to the lowest level which permitted the fibroblasts to live was associated with cessation of collagen deposition and resorption of collagen. The low pH level in an area of inflammation may also partly explain the collagen degeneration found in association with inflammation.

Pathologic changes in collagen are manifest as degenerative changes, and result from many causes, which include allergy, disturbance in metabolism, toxic influences, drugs, foreign proteins, endocrine imbalances, aging, and

reaction to excessive stress and inflammation. In addition, so-called collagen diseases exist, including serum sickness and scleroderma. The products of degeneration of collagen may be distinguished by staining reactions. The collagen first ceases to stain with acid dyes and stains with basic dyes. The structure of normal collagen is retained in this early stage of degeneration. Degenerative changes begin in the center of the fiber.[22] As the degenerative changes proceed, acid-staining collestin is formed. The collagen disintegrates into coarse, irregular masses or blocks. A more advanced degenerative product of collagen is the intensely basophilic-staining collacin. Atrophy and destruction of the collagenous fibers also occur in the chronic infective granulomatous processes such as tuberculosis and syphilis.

Little research has been reported with reference to collagen in periodontal tissues. Stafne and Austin[34] reported widened periodontal spaces in x-ray films of patients with acroselerosis and diffuse scleroderma. They found that the enlarged periodontal space was filled with loose vascular connective tissue. Ray[30] reported collagen degeneration in some gingival specimens from diabetics. The change in collagen was associated with inflammation, which made it difficult to evaluate the significance of the findings. Orban, Martin, and Helm,[25] in a case of scorbutus, demonstrated an increase in the formation of collagenous fibers in the gingiva when the scurvy improved.

In the reported cases of diffuse atrophy of the periodontal tissues, hematoxylin-eosin stains and in a few cases, silver stains, have been used to study collagen change. Collagenous degeneration has been diagnosed by the finding of dearrangement of the fibers rather than by staining reactions. Fixation of tissues from the jaws is often delayed in autopsy material. Uneven, inadequate fixation may also result from poor penetration of the fixative into these dense tissues. One portion of a specimen may be fixed and show cell details, while another portion of the same jaw may be inadequately fixed and the artefact which results might be misinterpreted as degenerative change. Material containing teeth requires a longer period of decalcification than ordinary bone, and sections including teeth are often uneven in thickness. The above technical factors are emphasized because they are important in the evaluation of collagen stains of jaw sections.

Various techniques for silver stains were tried. The best results were obtained with Rio Hortega's triple impregnation, but the silver stains were all uncertain in their action on decalcified and celloidin-embedded material[16] and no reliable color reaction indicating collagen degeneration could be established.

Heidenhain's modification of Mallory's connective tissue stain seemed more dependable and was utilized for a large number of sections both from jaws and joints, including experimental and control animals. Even with this method it proved to be very difficult to differentiate between slight degree of artefact and early collagen degeneration, so at least 10 to 15 sections from each specimen should be stained and compared before an interpretation of details is attempted. These technical hazards are discussed because they offer some explanation of the wide range of different findings and opinions en-

countered in the literature, although the main source of error in histological reports on periodontosis and systemic disease has been the lack of acceptable control material.

This investigation of the collagen fibers did not show any difference between the experimental and the control animals, neither in sections from joints nor in sections from jaws and teeth. The main variable factor, with reference to periodontal disease, which is present in man and absent in monkeys, is variations in the personal oral hygiene. The literature generally points out that the oral hygiene is poor in patients with far-advanced tuberculosis.[12, 35] If tuberculous human beings have a higher incidence of and a more severe periodontal disease than healthy individuals, it is probably on the basis of impaired oral hygiene rather than a change in the resistance of the periodontal tissues.

SUMMARY

1. No periodontal changes were observed that could be attributed to tuberculosis in 10 rhesus monkeys.

2. No indication of collagen changes was noted in sections from jaws and joints.

3. No observations substantiated the theory of lowered periodontal resistance in tuberculosis.

4. Any increase in incidence of periodontal disease associated with tuberculosis must be produced by local factors of which poor oral hygiene appears to be the most significant.

5. Traumatic periodontal changes resembling diffuse atrophy or periodontosis were observed both in the experimental and in the control animals.

REFERENCES

1. Appleton, J. L. T., Jr.: Bacterial Infection, Philadelphia, 1933, Lea and Febiger.
2. Belding, L. J., and Belding, P. H.: An Evaluation of the Various Theories on the Etiology of Periodontoclasia, *D. Cosmos* **75**: 140, 1933.
3. Boyle, P. E., Bessey, O., and Wolbach, S. B.: Experimental Production of the Diffuse Alveolar Bone Atrophy Type of Periodontal Disease by Diets Deficient in Ascorbic Acid (Vit. C), *J. A. D. A.* **24**: 1768, 1937.
4. Brodsky, B. H.: Tuberculosis of the Mouth, *Ann. Dent.* **6**: 167, 1947.
5. Brody, H.: Influence of Pulmonary Tuberculosis on the Diseases of the Mouth, *D. Cosmos* **64**: 407, 1922.
6. Bryan, A. W.: Progress in the Recognition of the Etiologic Factors in Periodontal Disease, *J. Periodont.* **10**: 25, 1939.
7. Burket, L. W.: Oral Medicine. Philadelphia, 1946, Lippincott Co.
8. Cahn, L. R.: Observations on the Effect of Tuberculosis on the Teeth, Gums and Jaws, *D. Cosmos* **67**: 479, 1925.
9. Cipes, B. J.: Oral Hygiene in Relation to Tuberculosis, *D. Cosmos* **68**: 50, 1926.
10. Citron, J.: Zur Frage der internistischen Aetiologie der Paradentose, *Zahnarzt. Rund.* **37**: No. 32, 1928.
11. Fish, W. E.: Surgical Pathology of the Mouth, London, 1948, Sir Isaac Pitman and Sons.
12. Franke, J.: Dystrophische Paradental Disposition, *Deutsche Zahnarzt. Zeit.* **3**: 505, 1948.
13. Gardner, W. M.: Teeth of the Tuberculous, *J. Outdoor Life* **23**: 77, 1926.
14. Goadby, K.: Diseases of the Gums and Oral Mucous Membrane, ed. 4, London, 1931, Oxford University Press.
15. Goldman, H. M.: Periodontosis in the Spider Monkey, *J. Periodont.* **18**: 34, 1947.
16. Gomori, G.: Effect of Certain Factors on Results of Silver Impregnation of Reticulum Fibers, *Am. J. Path.* **15**: 493, 1939.
17. Gottlieb, B.: Zur Aetiologie und Therapie der Alveolarpyorrhoe, *Ztschr. Stomatol.* **18**: 59, 1920.

18. Gruber, I. E.: The Condition of the Teeth and the Attachment Apparatus in Tuberculosis, *J. D. Res.* **28**: 483, 1949.
19. Hass, G., and McDonald, F.: Studies of Collagen. I and II, *Am. J. Path.* **16**: 525, 1940.
20. Hill, T. J.: Oral Pathology, ed. 4, Philadelphia, 1949, Lea and Febiger.
21. Kramer, C. S.: Osseous Trophic Changes in the Jaws and Palate in the Tuberculous, *J. A. D. A.* **12**: 1117, 1925.
22. McLeod, J. M. H., and Muende, I.: Pathology of the Skin. New York, 1946, Paul B. Hoeber.
23. Maximow, A.: Development of Argyrophile and Collagenous Fibers in Tissue Cultures, *Proc. Soc. Exper. Biol. & Med.* **25**: 439, 1928.
24. Miller, S. C.: Textbook of Periodontia, ed. 2, Philadelphia, 1947, The Blakiston Co.
25. Orban, B., Martin, W. B., and Helm, R. M.: Histopathologic Investigation of a Case of Scorbutic Gingivitis, *J. Periodont.* **18**: 95, 1947.
26. Orban, B., and Weinmann, J. P.: Diffuse Atrophy of the Alveolar Bone (Periodontosis), *J. Periodont.* **13**: 31, 1942.
27. Ordene, N. M.: Dental Aspects of Tuberculosis, *N. Y. State D. J.* **14**: 300, 1948.
28. Parma, C.: Histopathology of Periodontosis, *J. D. Res.* **27**: 766, 1948.
29. Ramfjord, S.: Effects of Acute Febrile Diseases on the Periodontium of Rhesus Monkeys With Reference to Poliomyelitis, *J. D. Res.* **30**: 615, 1951.
30. Ray, H. G.: A Study of the Histopathology of the Gingiva in Patients With Diabetes Mellitus, *J. Periodont.* **19**: 128, 1948.
31. Rhein, M. L.: Etiological Classification of Pyorrhea Alveolaris, *D. Cosmos* **36**: 779, 1894.
32. Roddy, J. A., Funk, E. H., and Kramer, D. W.: Pyorrhea Alveolaris, *N. Y. Med. J.* **104**: 433, 1916.
33. Schmuziger, P.: Primare Mundschleimhaut Tuberculose, *Schw. Monat. f. Zahn.* **55**: 1087, 1945.
34. Stafne, E., and Austin, L.: A Characteristic Dental Finding in Acrosclerosis and Diffuse Scleroderma, *J. Orthodontics* **30**: 25, 1944.
35. Tanchester, D., and Sorrin, S.: Dental Lesions in Relation to Pulmonary Tuberculosis, *J. D. Res.* **16**: 69, 1937.
36. Weinmann, J. P., and Sicher, H. D.: Bone and Bones, St. Louis, 1947, The C. V. Mosby Co.
37. Wissler, H.: Die Primare Mundschleimhaut-Tuberculose, *Schw. Monat. f. Zahn.* **54**: 905, 1944.
38. Wolbach, S. B.: Controlled Formation of Collagen and Reticulum. A Study of the Source of Intercellular Substance in Recovery From Experimental Scorbutus, *Am. J. Path.* **9**: 689, 1933.

STUDIES IN PERIODONTAL DISEASE

MAXWELL KARSHAN, BENJAMIN TENENBAUM, DANIEL E. ZISKIN AND ROSLYN FRIEDLAND*

From the Department of Biochemistry, School of Dental and Oral Surgery, and College of Physicians and Surgeons, Columbia University, New York, N. Y.

IN OUR previous blood studies[1, 2] of patients with periodontal disease involving alveolar bone loss, our results suggested that some systemic condition operated as one of the contributing factors in some of the subjects studied. This conclusion was based on the finding that differences were found between a control group and a group with periodontal disease in certain blood constituents and characteristics, such as vitamin C, alkaline phosphatase, sugar, total serum protein, serum globulin, cholesterol, leukocyte count and erythrocyte sedimentation rate. Other investigators have reported higher than normal values for total calcium, total cholesterol, uric acid, sugar and local phosphatase (phosphatase in gingival blood) in various percentages of cases of periodontal disease in which loss of bone was present. Lower than normal values were reported for vitamin C, erythrocyte count and CO_2-combining power in such cases. More recently, Seidler, Miller, and Wolf,[3] in an investigation of patients with "precocious advanced alveolar bone destruction," reported serum calcium and cholesterol values that ranged toward the upper limit of normality, somewhat lowered serum phosphatase values, flattened glucose tolerance curves and reductions in specific dynamic action of protein and basal metabolic rate.

In an extension of our investigation of the etiology of periodontal disease involving alveolar bone loss the following studies were carried out: comprehensive dental examination, blood chemistry, blood count, dietary record, determination of neutral 17-ketosteroids in urine, physical examination, basal metabolism, Kline test, and evaluation of ovarian function. The results of the study of ovarian function will be reported later.

EXPERIMENTAL

Dental Examination.—The dental examination included the following: full mouth series of roentgenograms, Kodachrome photographs of the gingivae, measurements of the depth of the pockets around each tooth, alginate impression for study models, and a biopsy of the interdental gingival papilla between the upper left canine and first premolar teeth for histological study. A report on this part of the investigation has been published elsewhere.[4]

In our two previous investigations, all cases were classified as periodontoclasia. On the basis of the more detailed dental examination, these cases and

This investigation was supported (in part) by a research grant from the National Institute for Dental Research, of the National Institutes of Health, Public Health Service, and in part by a grant from the Roche Anniversary Foundation. Presented at the general meeting of the International Association for Dental Research at French Lick, Indiana, on March 24, 1950 (J. D. Res. 29: 660, 1950).

Received for publication, August 9, 1951.

*Now at Ohio State University, College of Dentistry.

all subsequent ones have been divided into three groups as follows: Group 1—periodontosis* (or primary periodontosis according to Hill[5]); Group 2—periodontitis complex* (or secondary periodontosis according to Hill[5]); Group 3—advanced cases of periodontitis simplex.* A typical case in each group is shown in Figs. 1, 2, and 3. Patients in Groups 1 and 2 were predominantly females, whereas those in the third group were approximately equally divided between males and females.

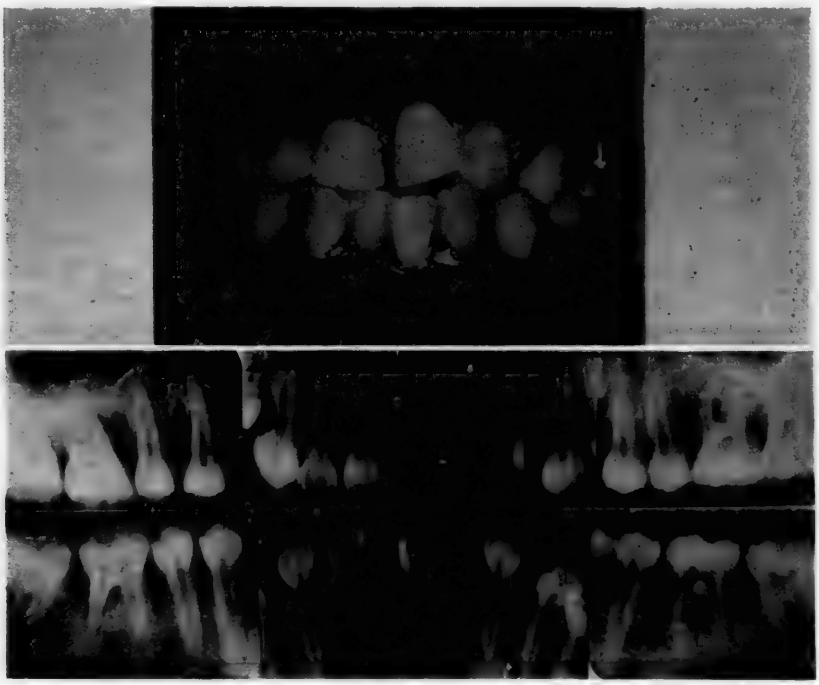

Fig. 1.—Periodontosis (Primary Periodontosis). Case 116: female, age 14. The gingiva was pigmented. A few papillae were hypertrophied and glossy. No calculus was present. The left first incisor was protruded and rotated. There were open contacts between many of the upper anterior teeth. Roentgenograms revealed marked loss of alveolar bone in the anterior region in both the maxilla and mandible. There was also loss of alveolar bone in the form of an arc around the left first molars. The beginning of a similar type of bone loss may be seen around the right molars. In cases of this type local factors were either absent or not prominent and apparently did not account for the severe loss of alveolar bone. These observations were rather constant in this group in individuals under 25 years of age.

Blood Chemistry and Blood Counts.—Inasmuch as the cases were divided into three groups, it seemed desirable to re-evaluate our previous blood data on this basis and furthermore to incorporate the blood data from 50 new periodontosis cases with that of our two previous reports.[1, 2] The total number of cases including controls was 229. There were 54 cases in the control group (individuals free from periodontal disease); 77 cases in the periodontosis group; 59 cases in periodontitis complex group; and 39 advanced cases of periodontitis simplex. The methods used are described in a previous publica-

*This is the terminology used in the classification of periodontal disease of the American Academy of Periodontology.

tion.[2] The results are summarized in Table I. The figures represent the percentage of cases with values outside the normal ranges as reported in the literature. The deviations of individual cases from these normal ranges, in general, were small. Blood constituents or characteristics for which differences were found between the control and each of the periodontal groups include vitamin C, glucose, globulin, albumin, calcium-ion, alkaline phosphatase, red blood cell sedimentation rate, red blood count, white blood count, and neutrophils. For

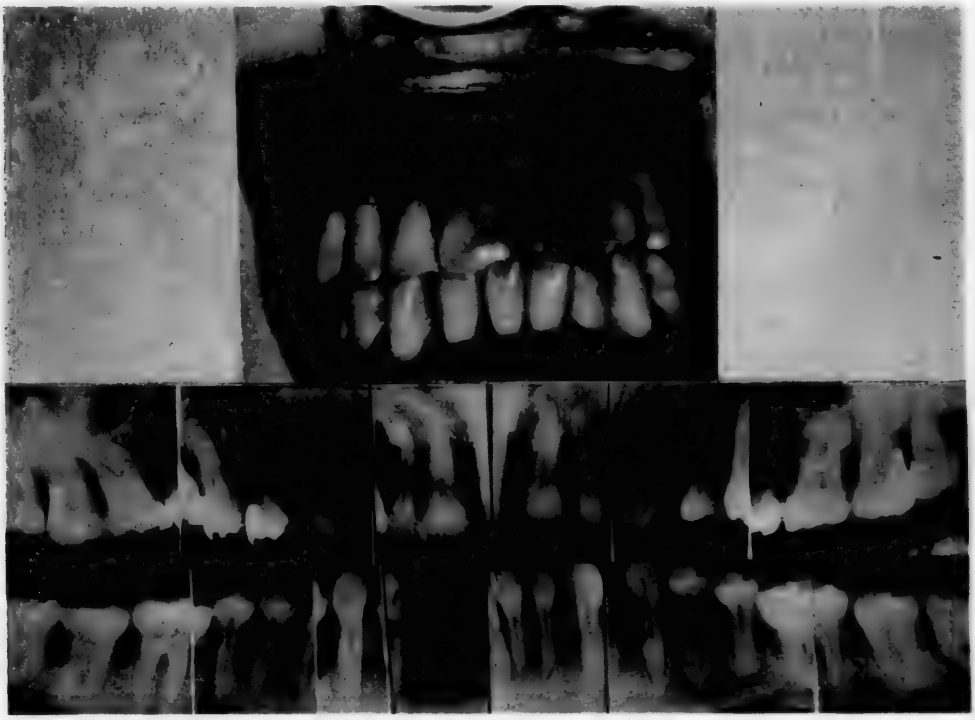

Fig. 2.—Periodontitis complex (Secondary Periodontosis). Case 40: female, age 32. The gingiva had lost its stippling and had a purplish tinge. A slight amount of calculus was present. Papillae were blunt and appeared glossy and spongy. Diastemata were present between many of the anterior teeth. The upper right first incisor was slightly elongated. The maxillary anterior teeth were protruded. Roentgenograms showed an almost complete loss of alveolar bone in both arches with the exception of the mandibular bicuspid aren. Many of the teeth showed second and third degree mobility. In this group local factors were more prominent than in the first group.

the other blood constituents studied, no appreciable differences were found between the control and periodontal disease groups, although deviations from the normal ranges were obtained for both groups. This was the case for total calcium, inorganic phosphate, sodium, chloride, total protein, total cholesterol, free cholesterol, nonprotein nitrogen, urea, uric acid, carbon dioxide capacity, hemoglobin, vitamin A, and carotene.

Dietary Record.—A dietary record was made of the total amount of food eaten during a period of one week. The intake of some of the essential food factors was calculated from the information thus obtained. This was done for

TABLE I

DEVIATIONS FROM NORMAL RANGES IN THE CONTROL AND EXPERIMENTAL GROUPS

	PERCENTAGE OF CASES WITH VALUES OUTSIDE THE NORMAL RANGE							
	CONTROL GROUP		PERIODONTOSIS GROUP		PERIODONTITIS COMPLEX GROUP		PERIODONTITIS SIMPLEX GROUP	
	54 CASES MEAN AGE 33.5		77 CASES MEAN AGE 27.3		59 CASES MEAN AGE 39.1		39 CASES MEAN AGE 48.8	
	ABOVE	BELOW	ABOVE	BELOW	ABOVE	BELOW	ABOVE	BELOW
Vitamin C, in plasma		15.0		26.3		42.5		41
Glucose in whole blood	1.8		16.1		15.1		22.5	
Globulin in serum	3.2		28.8		20.6		28.6	
Albumin in serum		3.2		14.9		10.3		21.4
Calcium-ion in serum		5.7		13.5		12.1		8.1
Alkaline phosphatase in serum	0		8.6		13.8		20.0	
R.B.C. sedimentation rate	11.5		42.5		41.5		50.0	
Red blood count	3.1	3.1	18.8	10.7	6.7	16.6	11.8	17.7
White blood count	0		14.7		6.5		11.3	
Neutrophils	0		13.4		9.7			11.5

a control group of 22 individuals free from periodontal disease and for 24 individuals in the periodontosis (primary periodontosis) and periodontitis complex (secondary periodontosis) groups. By comparing the amount of food eaten with the recommended daily allowances of the Food and Nutrition Board of the National Research Council,[6] the percentage of cases showing low intakes was calculated. A summary of some of the results is given in Table II. Inasmuch as essentially similar results were obtained in primary and secondary periodontosis, the results of these two groups are combined. It may be noted that a large percentage of both control and periodontosis cases do not measure up to the standards set by the Food and Nutrition Board for calories, protein, calcium, phosphorus, iron, thiamin, riboflavin, and niacin. Furthermore, there is no fundamental difference between the control and periodontosis groups. However, it should be emphasized that the recommended dietary allowances are admittedly higher than average requirements and that the deviations from these allowances, in general, were small. The results for vitamins A and C were in striking contrast to the other dietary constituents. With few exceptions, adequate amounts of these two vitamins were ingested by both groups.

TABLE II

PERCENTAGE OF CASES SHOWING LOW FOOD INTAKE*

	CONTROL GROUP 22 CASES	PERIODONTOSIS GROUPS 24 CASES
Calories	45	48
Protein	23	24
Calcium	73	70
Phosphorus	55	58
Iron	36	45
Thiamin	36	21
Riboflavin	45	50
Niacin	41	41

*Based on the dietary allowances recommended by the Food and Nutrition Board, National Research Council.

Neutral 17-ketosteroids in urine.—Adrenocortical function was studied by determining the urinary neutral 17-ketosteroid excretion.[7] Results are presented only for females in the series. There were 20 cases in the control group and 33 in the periodontosis (primary periodontosis) and periodontitis complex (secondary periodontosis) groups. The results are given in Table III. The mean excretion for the control group was 11.3 mg. per 24 hours as compared with 9.8 mg. per 24 hours for the periodontosis groups. The difference between the means divided by the standard error of the difference is 1.44, which indicates that there is no significant difference between the control and periodontosis groups. However, it is of interest to note that 30 per cent of the cases in the control group are below 9.3 mg. per 24 hours (mean for females between 17 and 64 years[8]), whereas 52 per cent of the cases in the periodontosis groups are below this value.

*Physical Examinations**.—Physical examinations were made and detailed case histories were taken of the patients in the periodontosis groups. Of 50

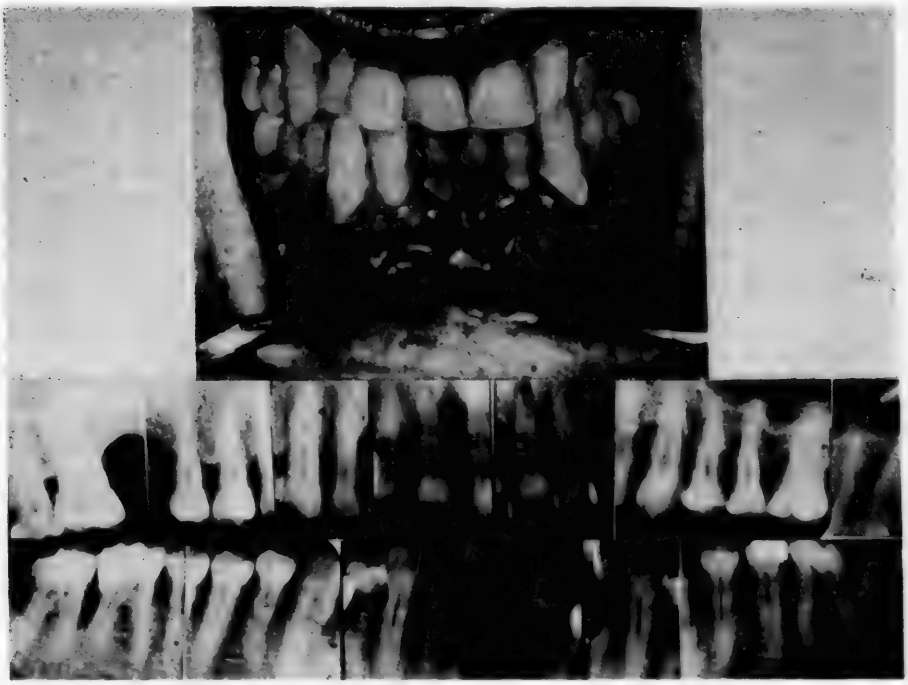

Fig. 3.—Advanced case of periodontitis simplex. Case 52: male, age 58. The gingiva was red, hypertrophied and glossy especially around the lower anterior teeth. This patient has received local treatment in the clinic for 10 years. Although this photograph was taken after a recent prophylaxis, calculus deposits were nevertheless present. In several areas pus could be expressed from the gingival crevices upon finger pressure. The anterior teeth did not appear elongated or protruded although they were in traumatic occlusion. There were no open contacts. Roentgenograms revealed a horizontal type of alveolar bone loss that was more marked in the anterior region. The teeth were relatively firm. In this group the teeth showed marked mobility only in the terminal stages.

*The physical examinations were made by Dr. Frederick K. Heath of the Department of Medicine.

TABLE III

MEAN VALUES FOR NEUTRAL 17-KETOSTEROIDS IN URINE FOR FEMALES IN CONTROL AND PERIODONTOSIS GROUPS

	NO. OF CASES	MEAN AGE	NEUTRAL 17-KETOSTEROID MG. PER 24 HOURS	PERCENTAGE OF CASES WITH VALUES BELOW 9.3 MG. PER 24 HOURS
Periodontosis Group	20	32	11.3	30
Control Group	33	29	9.8	52
Difference between means divided by the standard error of the difference.			1.44	
Odds that the difference between the means is not due to chance.			6 to 1	

cases, 21 gave essentially negative results. A number of apparently unrelated conditions were revealed in the remainder of the group. These included the following:

Systolic murmur (or mitral stenosis) (6 cases), diabetes (3 cases), hay fever (3 cases), mild hyperthyroidism (2 cases), intermittent dyspepsia (2 cases), and seborrheic dermatitis (2 cases). The following were found (each in one case): enlarged liver and tender spleen, amebic colitis, recurring peritoneal abscesses, hysterectomy because of fibroid tumor, recurring maxillary sinusitis, Hodgkin's disease (chronic inflammatory enlargement of the lymph nodes, enlargement of spleen and often of liver and kidneys), cervical lymph node tuberculosis, history of syphilis and gonorrhea, cystocele (hernia of the bladder) and palpable right kidney, and thickening and roughening of the skin and moderate hirsutism. It is thus clear that these examinations did not show a consistent pattern in the periodontosis patients.

Basal Metabolism and Kline Test.—No difference was found between the control and periodontosis groups in the basal metabolic rate. The Kline test was negative in all cases.

CONCLUSIONS AND SUMMARY

Blood studies of patients with periodontosis (primary periodontosis), periodontitis complex (secondary periodontosis) and advanced periodontitis simplex showed that deviations from normal ranges were present in a greater percentage of patients in each of these groups than in a control group free from periodontal disease. This was the case for vitamin C, glucose, globulin, albumin, calcium-ion, alkaline phosphatase, red blood cell sedimentation rate, red blood count, white blood count and neutrophiles. No appreciable differences were found between the control and periodontal disease groups for certain other blood constituents. The fact that more deviations were found in the groups with periodontal disease than in the control group suggests that one or more systemic conditions are associated with these diseases in many of the subjects studied. The relationship may be one of effect rather than of cause in the cases of phosphatase, erythrocyte sedimentation rate and leukocyte count. Inasmuch as deviations from the normal were obtained for several apparently unrelated blood constituents, it does not appear that a common systemic condition operates as a modifying or predisposing factor in these condi-

tions. No clear distinction in blood chemistry was found between the three groups with periodontal disease. This is of interest in view of the emphasis that is generally placed on local factors in the mouth as the major, if not the sole, cause of periodontitis simplex.

A record of the food intake over a period of one week indicated that a large percentage of the individuals in the control and periodontosis groups fell below the standards recommended by the Food and Nutrition Board of the National Research Council for calories, protein, calcium, phosphorus, iron, thiamin, riboflavin and niacin. However, there was no fundamental difference between these groups. On the other hand with few exceptions, the intakes of vitamins A and C were adequate for both groups. The fact that plasma vitamin C was low in a large percentage of the periodontosis cases in spite of an adequate intake of this vitamin may mean that there was some interference in the utilization of this vitamin.

Neutral 17-ketosteroid output in the urine was determined to study adrenocortical activity. The mean output was somewhat greater in the control than in the periodontosis groups but the difference between the means of the two groups is not statistically significant.

Physical examinations, including case histories did not reveal a common systemic condition responsible for periodontosis and periodontitis complex. A miscellaneous number of conditions were found in 58 per cent of the cases.

There was no difference between the control and periodontosis groups in the basal metabolic rate. The Kline test was negative in all cases.

REFERENCES

1. Tenenbaum, B., and Karshan, M.: Blood Studies in Periodontoclasia, *J. A. D. A.* **32**: 1372, 1945.
2. Karshan, M., Tenenbaum, B., Karlan, F. R., and Leonard, H. J.: Blood Studies in Periodontoclasia II, *J. D. Res.* **25**: 247, 1946.
3. Seidler, B., Miller, S. C., and Wolf, W.: Some Aspects of Precocious Advanced Alveolar Bone Destruction: Preliminary Report, *J. A. D. A.* **40**: 49, 1950.
4. Tenenbaum, B., and Karshan, M.: Clinical and Microscopic Study of the Gingivae in Periodontosis, *J. A. D. A.* **40**: 302, 1950.
5. Hill, T. J.: A Textbook of Oral Pathology, ed. 4, Philadelphia, 1949, Lea and Febiger, p. 255.
6. National Research Council, Reprint and Circular Series Number 129, Oct. 1948, p. 16.
7. Callow, N. H., Callow, R. K., and Emmons, C. W.: A Colorimetric Determination of Substances Containing the Grouping $—CH_2CO—$ in Urine Extracts as an Indication of Androgen Content (modified), *Biochemical J.* **32**: 1312, 1938.
8. Kenigsberg, S., Pearson, S., and McGavack, T. H.: The Excretions of 17-Ketosteroids. I. Normal Values in Relation to Age and Sex, *J. Clin. Endocrinology* **9**: 426, 1949.

IDENTIFICATION OF A PROTEOLYTIC ENZYME OF THE PERIODONTAL POCKET

GENEVIEVE D. ROTH AND HUGH I. MYERS

The University of Kansas City School of Dentistry, Kansas City, Mo.

THE principal fibers of the periodontal membrane are white fibrous connective tissue, collagenous in nature. Box,[1] in considering the pathogenesis of periodontal diseases stated, "the findings suggest that extra-cellular proteolytic enzymes are involved. The cuticle is attacked, and, with the penetration to the collagen fibres at the base of the epithelial attachment, these fibres are disorganized. . . . The destructive changes, localized and widespread, observed in the deeper fibre bundles and the extensive lesions in the periodontal membrane are due to the action of a fairly powerful necrotizing agent."

Miller,[2] in discussing pocket extension, suggested that, "the connective tissue fibers of the periodontal membrane must become detached from their anchorage in the cementum by trauma, inflammation, or some other local irritant."

Collagen is insoluble in the usual protein solvents. It can be destroyed by an enzyme, collagenase, which has been purified and isolated from the other toxins produced by *Cl. welchii,* type A. It acts over a pH range of 6.0 to 7.5.[3, 4, 5, 6, 7, 8]

Engel[9] and Engel, Ray and Orban[10] have shown the compatibility between collagenase and oral environment.

Based on the above rationale, this study was undertaken to determine the relationship between collagenase and periodontal disease. The preliminary data herein presented are offered to indicate that collagenase activity can be demonstrated in material cultivated from the periodontal pocket.

MATERIALS AND METHODS

Sterile paper points were introduced into the periodontal pockets of 69 subjects and each point transferred to a fluid medium consisting of 1 ml. horse serum, a small piece of rat kidney, and 3 ml. distilled water.[11] A thin piece of sterile collagen paper, approximately 2 mm. by 10 mm., prepared from rat tail tendon according to the method of Oakley, Warrack, and van Heyningen[4] was placed in each tube and the preparations incubated anaerobically at 37° C. Nine controls with sterile paper points in the fluid medium-kidney-collagen paper environment were set up.

After 21 days all tubes were opened and the remaining collagen, kidney and paper point were placed on a watch glass for study. Three pieces of kidney, one from a control tube, one from a negative experiment and one from a positive experiment were sectioned at 6 microns and stained with hematoxylin-eosin and Van Gieson's stains.

Received for publication, April 19, 1951.
Revised by authors, September 24, 1951.

OBSERVATIONS

The results with the series of 69 tests and 9 controls are shown in Table I. The section of kidney from a tube in which collagen was dissolved showed intact kidney tubules, but total destruction of the supporting stroma. The kidney, in a tube where the collagen paper was not lysed, showed an anemic infarct with the connective tissue elements intact, essentially the same condition as was found in a section of the control.

DISCUSSION

It is apparent from the literature cited, recognized investigators concede the destruction of collagen plays a role in periodontal disease. The preliminary data presented in this report indicate that collagenase activity is demonstrable in material cultivated from periodontal pockets. To date, however, the source of the enzyme or enzymes involved has not been determined.

TABLE I

	NUMBER OF TESTS	COMPLETE LYSIS	PARTIAL LYSIS	NO LYSIS
Collagen (Experimental)	69	15	11	43
Collagen (Control)	9	1*	1	8
Kidney—Gross (Experimental)	69	8	20	41
Kidney—Gross (Control)	9	1*	0	8

*Contaminated.

REFERENCES

1. Box, Harold K.: New Aspects of Periodontal Research, *J. Canad. D. Assoc.* **13**: 3. 1947.
2. Miller, Samuel C.: Textbook of Periodontia, Philadelphia, 1950, The Blakiston Company.
3. Macfarlane, R. G., and Mac Lennan, J. D.: Toxemia of Gas Gangrene, *Lancet* **249**: 328, 1945.
4. Oakley, C. L., Warrack, G. Harriet, and van Heyningen, W. E.: The Collagenase (κ Toxin) of Cl. welchii Type A, *J. Path. and Bact.* **58**: 229, 1946.
5. Bidwell, E., and van Heyningen, W. E.: The Biochemistry of the Gas Gangrene Toxins. 5. The κ Toxin (Collagenase) of Clostridium welchii, *Biochem. J.* **42**: 140, 1948.
6. Bidwell, E.: The Inhibition by Normal Horse Serum of (a) Trypsin and (b) a Proteolytic Enzyme of Clostridium welchii Type B (λ enzyme), Proc. Biochem. Soc., *Biochem. J.* **44**: 1494.
7. Bidwell, E.: The κ Toxin (Collagenase) of Clostridium welchii. 2. The Effect of Alkaline pH and Heat, *Biochem. J.* **44**: 28, 1949.
8. Bidwell, E.: Proteolytic Enzymes of Clostridium welchii, *Biochem. J.* **46**: 589, 1950.
9. Engel, Milton B.: The Softening and Solution of the Dentin in Caries, *J. D. Res.* **28**: 646, 1949.
10. Engel, Milton B., Ray, Harold G., and Orban, Balint: The Pathogenesis of Desquamative Gingivitis: A Disturbance of the Connective Tissue Ground Substance, *J. D. Res.* **29**: 410, 1950.
11. Noguchi, H.: A System of Bacteriology in Relation to Medicine, Privy Council, Medical Research Council **9**: 91, 1931.

INHIBITION OF ORAL SPIROCHETES BY ANTIBIOTIC AGENTS IN VITRO

ROBERT J. FITZGERALD AND EDWARD G. HAMPP*

National Institute of Dental Research, National Institute of Public Health Service, Bethesda, Md.

A NUMBER of antibiotics have been employed for treatment of Vincent's infection (ulcerative gingivo-stomatitis). These include penicillin, streptomycin, and aureomycin.[1, 2, 3] In view of the fact that the precise etiology of this disease is still open to debate, it seemed of interest to investigate the effects of various antibiotics with this condition. The present phase of the investigation deals with in vitro inhibition of the growth of several strains of *Borrelia vincentii* and small oral treponemes.

EXPERIMENTAL METHODS

Cultures.—Three strains of *Borrelia vincentii* and three strains of small oral treponemes were employed in this study. *B. vincentii* strains N9 and N19 and the small oral treponemes FM and MRB have been the subject of earlier studies in which their morphological characteristics were described.[4, 5] These strains and the previously undescribed small oral treponeme strain NF were isolated from clinical cases of ulcerative gingivo-stomatitis. Strain EH was isolated from healthy gingiva and is indistinguishable from the other strains of *B. vincentii* in cultural and morphological characteristics. Stock cultures of the various organisms were carried by monthly transfer in 1.5 per cent agar stabs of the medium described below.

Media.—The agar and broth used in this study are modifications[6, 7] of Huntoon's "hormone" medium.[8] In contrast to a recent report[9] we found such a broth medium to support adequately reproducible growth of the oral spirochetes when used in volumetric flasks filled almost to the top and rubber stoppered. Equally good results can be obtained with 10 ml. amounts in stoppered 15 by 125 mm. test tubes providing small amounts of agar are added to the medium.

It has been our experience, however, that "poor" batches of medium sometimes result if the recipe is not strictly followed. For this reason, the preparation of this medium as modified by Hampp[7] is described in detail.

MODIFIED HUNTOON'S HORMONE BROTH

Ingredients for Basal Medium:

Fresh veal hearts (freed of fat and connective tissue)	1000 Gm.
Distilled water	2000 ml.
Thiopeptone (BBL)	20 Gm.
Sodium Chloride	10 Gm.
Whole Fresh Eggs	2 eggs

Directions.—(1) Veal hearts, freed of fat and connective tissue, are finely ground in a meat grinder. (2) Add water, mix well and add the remaining ingredients. (3) Heat slowly, with constant stirring at 70° C. (4) Adjust

Received for publication, June 12, 1951.

*Fellow, Research Council, American Dental Association.

pH to 7.4 with 0.1N NaOH and heat in Arnold Sterilizer for 2 hours at 100° C. (5) Filter through glass wool (cloth or vegetable fiber filters are not satisfactory). (6) Readjust pH to 7.6 and heat again in Arnold sterilizer 30 minutes at 100° C. (7) Store overnight in cold room. (8) Skim off congealed fat and filter broth through glass wool while cold. (9) Sterilize by heating in Arnold sterilizer for 60 minutes at 100° C. on two successive days. (10) Using a sterile glass wool filtering assembly dispense in sterile bottles in 400 ml. amounts. (11) Heat to 100° C. for 30 minutes in Arnold sterilizer. (12) Before use the clear basal broth is decanted or pipetted free of the fine precipitate which settles out on standing and heated to 100° C. (13) Add 7.5 ml. melted modified Huntoon's Agar for each 100 ml. of broth. (14) Allow to cool and just before dispensing add 10 ml. of a sterile solution of human serum albumin* and 10 ml. of a sterile 1 per cent solution of glutathione for each 100 ml. of broth. (15) Using aseptic precautions the medium is dispensed in 10 ml. amounts into sterile cotton stoppered 15 by 125 mm. test tubes.

The final pH of the medium is 6.8 to 7.0 without further adjustment. The final concentration of agar in the medium is approximately 0.075 per cent. This was selected by previous trials as the concentration at which the medium supported maximal growth in test tubes and yet remained sufficiently fluid to permit adequate mixing of drugs and supplements.

Drug Solutions.†—Wherever possible the drugs were made up in a series of sterile aqueous stock solutions of sufficient strength that 0.2 ml. gave the desired final concentration when mixed with 10 ml. of medium.

Drugs not sufficiently soluble in water were dissolved in the minimum amount of ethyl alcohol or propylene glycol and subsequently diluted with distilled water. Suitable controls were included in these tests to rule out the effects of the solvents on the observed results.

The drugs were added to the tubes first and the broth was then pipetted into the tubes to mix the drugs.

Inoculum.—The inoculum for each tube was 0.2 ml. of a 7-day-old modified Huntoon broth culture of the appropriate organism. These cultures were grown in 25 ml. volumetric flasks with no added agar. Following inoculation of the test tubes, the cotton stoppers were cut off level with the lip of the tube and sterile No. 1 rubber stoppers were inserted. All tests were incubated in air at 37° C.

Reading of Results.—Endpoints were determined by visual inspection as the least concentration of drug which completely inhibited growth at seven days. Readings were also made at shorter and longer intervals and checked by dark-field examination when visual readings were questionable.

*Squibb's "salt poor" concentrated solution of human serum albumin with no preservative was used here. Ascitic fluid usually permits even better growth but was not used because of the difficulty of getting a single supply adequate enough to use for all the tests. Inactivated rabbit or human serum may also be substituted in equal concentrations.

†The drugs used in this study and their sources were as follows: Aureomycin hydrochloride, furnished by Lederle Laboratories; bacitracin, furnished by Commercial Solvents Corporation; chloromycetin and tyrothricin, furnished by Parke, Davis and Co.; lupulon[10], furnished by the Western Regional Research Laboratories, U.S.D.A., notatin[11], furnished by the Boots Pure Drug Co., Ltd. All of the above substances were gifts of the respective organizations which are gratefully acknowledged. In addition the following commercially available drugs were used: streptomycin ($CaCl_2$ complex), crystalline Na penicillin G, and sulpharsphenamine.

RESULTS AND DISCUSSIONS

The results of the titrations of various drugs against some oral spirochetes in vitro are shown in Table I. The endpoints are expressed as the least amount of drug which prevents visible growth of the spirochetes after seven days' incubation. For convenience this is referred to as the M.E.C. (minimal effective concentration). With the exception of lupulon and notatin results are based on two or more tests. Aside from notatin all of the drugs tested exhibit an appreciable inhibitory effect on both the small oral treponemes and *B. vincentii.*

The test with notatin seems deserving of additional comment. This substance has been shown to be an enzyme, glucose oxidase, produced by *Penicillium notatum.*[11] In the presence of oxygen it converts glucose to gluconic acid. Its antibacterial action is due to the fact that it also produces equimolar amounts of hydrogen peroxide in this reaction. For purposes of the antispirochete tests with notatin, 0.5 per cent glucose was incorporated in the test medium. Although the medium used in these tests is admittedly designed to provide micro-aerophilic conditions for the growth of spirochetes there was apparently sufficient O_2 present for the enzyme to function and to permit some H_2O_2 production. This was demonstrated by inoculation of a parallel series of notatin tubes with a strain of *Staphylococcus aureus.* The inhibitory endpoint for *S. aureus* was 200 units per milliliter of notatin compared with 1000 units per milliliter or more for the oral spirochetes. The inhibitory endpoint remained the same for *S. aureus* even if the notatin-containing tubes were incubated at 37° C. for 5 days prior to inoculation with *S. aureus.* On the other hand the sensitivity of the oral spirochetes and *S. aureus* to hydrogen peroxide is of the same magnitude, ranging from 50 to 150 μg per milliliter in the modified Huntoon's broth. It is not possible at this stage to explain the significance of this apparent discrepancy. One obvious possibility is that the spirochetes may in some way inhibit the action of notatin so that the production of H_2O_2 is diminished.

Drug Resistance.—In all of the tests there was only one instance in which a drug-resistant strain appeared spontaneously. The small oral treponeme, FM, grew out after 12 days in a tube containing 100 μg per milliliter streptomycin. At this time no growth had appeared in succeeding tubes of the series down through the normal MEC of 2.0 μg per milliliter. In a subsequent titration this resistant sub-strain grew readily at the highest streptomycin concentration used, 2000 μg per milliliter. A subculture from this tube grew out at 10,000 μg per milliliter streptomycin but not at 50,000 μg per milliliter.

Attempts were made to induce resistance to some of the other antibiotics by transferring from the highest concentration permitting growth at 7 days to the next higher tube in the series. This amounted to doubling the drug concentration. These attempts were uniformly unsuccessful. However, they are revealing in another sense since they indicate the persistence in these tubes of sufficient amounts of the drugs to be effectively inhibitory for 7 days or more in these tests. This was true for penicillin as well as the other antibiotics tested.

TABLE I

EFFECTS OF VARIOUS DRUGS ON GROWTH OF ORAL SPIROCHETES

	BORRELIA VINCENTII STRAINS			SMALL ORAL TREPONEME STRAINS		
	EH	N19	N9	FM	MRB	NF
Hydrogen peroxide	50.0 μg/ml.*	50.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.
Sulpharsphenamine	100.0 μg/ml.	50.0 μg/ml.	50.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.	50.0 μg/ml.
Lupulon	40.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.	100.0 μg/ml.
Tyrothricin	5.0 μg/ml.	5.0 μg/ml.	15.0 μg/ml.	20.0 μg/ml.	15.0 μg/ml.	15.0 μg/ml.
Chloromycetin	0.25 μg/ml.	0.25 μg/ml.	0.75 μg/ml.	1.5 μg/ml.	0.5 μg/ml.	0.5 μg/ml.
Aureomycin	0.25 μg/ml.	1.0 μg/ml.	1.0 μg/ml.	0.75 μg/ml.	0.75 μg/ml.	0.75 μg/ml.
Streptomycin	5.0 μg/ml.	2.0 μg/ml.	10.0 μg./ml.	2.0 μg/ml.	5.0 μg/ml.	1.0 μg/ml.
Notatin**	1000 units/ml.	4000 units/ml.	>4000 units/ml.	1000 units/ml.	2000 units/ml.	4000 units/ml.
Bacitracin	0.05 units/ml.	0.05 units/ml.	0.25 units/ml.	0.25 units/ml.	0.25 units/ml.	0.25 units/ml.
Penicillin G.	0.05 units/ml.	0.05 units/ml.	0.05 units/ml.	0.05 units/ml.	0.05 units/ml.	0.05 units/ml.

*M.E.C. or least concentration at which no visible growth occurs after 7 days at 37°C.
**0.5% glucose added to medium.

Spirochetocidal Action of the Drugs.—Some rough attempts were made to distinguish between the inhibitory and lethal concentrations of the drugs for the oral spirochetes. Two general methods were used: (1) Dark-field examination for viability as evidenced by motility, and (2) subculture into drug free medium. In the case of penicillin, Penicillinase (Schenley) was also employed to inactivate the drug. By these means it was found that for streptomycin, aureomycin, chloromycetin and penicillin the lethal concentration at 7 days was between 2 and 4 times the inhibitory concentration. In the drug titration system employed this would mean a difference of one or two tubes. For hydrogen peroxide and lupulon the lethal and inhibitory endpoints were the same.

SUMMARY

A number of drugs were tested as inhibitors of growth of three strains of *B. vincentii* and three strains of small oral treponemes in vitro. Penicillin, bacitracin, chloromycetin, aureomycin, streptomycin, and tyrothricin inhibit growth of the oral spirochetes within concentration ranges that can be readily attained in the oral cavity. Sulpharsphenamine, hydrogen peroxide, and lupulon were also active. Under the test conditions employed notatin was relatively inactive.

Drug resistance could not be readily induced in the oral spirochetes and only one "spontaneously" resistant variant appeared. This was a small oral treponeme that was streptomycin resistant.

REFERENCES

1. Atkinson, H. F.: Observations on the Treatment of Over 100 Cases of Acute Ulcerative Gingivitis With Penicillin Pastilles, *Brit. D. J.* **79:** 317, 1945.
2. Rosenthal, S. L., and Winter, G. R.: Comparative Studies of Streptomycin and Penicillin in the Treatment of Infections of the Oral Mucosa, *J. D. Res.* **29:** 237, 1950.
3. Stewart, G. M., and Roth, L. N.: The Use of Aureomycin in Oral Infections, *J. A. D. A.* **40:** 563, 1950.
4. Hampp, E. G.: Comparative Study of Dark-Field and Stained Smear Techniques for Identification of Oral Spirochetes on the Basis of Morphologic Characteristics, *J. A. D. A.* **32:** 318, 1945.
5. Hampp, E. G.: Morphologic Characteristics of the Smaller Oral Treponemes and Borrelia vincentii as Revealed by Stained Smear, Dark-Field and Electron Microscopic Technics, *J. A. D. A.* **40:** 1, 1950.
6. Hampp, E. G.: A Method for Routine Isolation and Cultivation of the Smaller Oral Treponemes, *J. A. D. A.* **30:** 1066, 1943.
7. Hampp, E. G., Scott, D. B., and Wyckoff, R. W. G.: Morphologic Characteristics of Certain Cultured Strains of Oral Spirochetes and Treponema pallidum as Revealed by the Electron Microscope, *J. Bact.* **56:** 755, 1948.
8. Huntoon, F. M.: "Hormone" Medium, Simple Medium Employable as Substitute for Serum Medium, *J. Inf. Dis.* **23:** 169, 1918.
9. Rosebury, T., Macdonald, J. B., Ellison, S. A., and Engel, S. G.: Media and Methods for Separation and Cultivation of Oral Spirochetes, *Oral Surg., Oral Med. & Oral Path.* **4:** 68, 1951.
10. Salle, A. J., Jann, G. J., and Ordanik, M.: Lupulon—An Antibiotic Extracted From the Strobiles of Humulus lupulus, *Proc. Soc. Exp. Biol. & Med.* **70:** 409, 1949.
11. Coulthard, C. E., et al.: Notatin: An Antibacterial Glucose-Aerodehydrogenase From Penicillium notatum (Westling) and P. resticulosum sp. nov., *Biochem. J.* **39:** 24, 1945.

PHOSPHATASE AND THE ORAL ACTINOMYCES

J. J. ENNEVER AND B. W. WARNER

University of Kansas City, School of Dentistry, Kansas City, Mo.

IT IS quite probable that no single factor is entirely responsible for the formation of dental calculus; more likely, an interplay of several is involved. In this regard, the significance of bacterial activity has been relatively well established but, as yet, the mechanisms have not been clarified.

The study here presented has been concerned with the phosphatase activity of oral actinomyces and their possible role in the formation of calculus deposits. The work was based upon a preliminary report of this nature by Citron.[3]

LITERATURE

Reviews embracing the various schools of thought concerning calculus formation have been presented by Prinz,[9] Tenenbaum and Karshan,[11] Rosebury,[10] Jacobsen and Kesel,[6] and Hodge and Wah Leung.[5]

Naeslund[8] was among the first to observe that dental calculus contained large numbers of long, filamentous microorganisms and indicated that some of these elements were actinomyces. He suggested that they were involved in the formation of calculus by attaching to the tooth surface in the form of a tenacious meshwork and causing precipitation of inorganic salts into this stroma by proteolysis. Bibby[1] showed that in vitro calculus formation did not occur in the absence of bacterial activity. However, many have questioned Naeslund on the proteolytic capacity of the actinomyces. Rosebury[10] wrote, "An alternative explanation, involving the action of phosphatases, may perhaps be substituted for the doubtful part of Naeslund's theory. The evidence on this point, however, is incomplete and hardly more than suggestive." Shortly thereafter, Citron[3] demonstrated that two strains of *Actinomyces israeli* possessed phosphatase activity.

MATERIALS AND METHODS

Eleven strains of actinomyces were isolated from clinically normal mouths according to the procedure of Ennever, Robinson, and Kitchin.[4] Each was examined for phosphatase activity by the culture plate indicator method of Bray and King[2] following four days anaerobic incubation with five per cent carbon dioxide at 35° C. The phenolphthalein phosphate used was prepared as reported by King.[7]

RESULTS

The eleven strains of actinomyces studied showed marked phosphatase activity. The method did not permit quantitation of the activity observed.

Received for publication, June 22, 1951.

DISCUSSION

Naeslund[8] believed that the actinomyces exerted a dual influence on the formation of dental calculus, physically affording a microbial stroma attached to the dental surfaces, and chemically inducing the precipitation of inorganic salts into this framework by proteolysis. The physical concept has been well received, but the proteolytic potential of the actinomyces has been questioned. The initial report by Citron[3] and the results of this present study give credence to Rosebury's[10] suggestion that the chemical influence might be phosphatase activity. However, the spectrum of phosphatases produced by the actinomyces remains unknown, since this investigation was limited to the observable response of phenolphthalein phosphate, as substrate.

SUMMARY

1. Eleven strains of actinomyces, isolated from clinically normal mouths, were positive for phosphatase activity.

2. The results confirmed the earlier preliminary study of Citron and substantiated Rosebury's suggestion.

REFERENCES

1. Bibby, B. G.: The Formation of Salivary Calculus, *D. Cosmos.* **77**: 668, 1935.
2. Bray, J., and King, E. J.: The Phosphatase Reaction as an Aid to the Identification of Micro-organisms Using Phenolphthalein Phosphate as Substrate, *J. Path. and Bact.* **55**: 315, 1943.
3. Citron, S. R.: The Role of Actinomyces israeli in Salivary Calculus Formation, *J. D. Res.* **24**: 87, 1945.
4. Ennever, J., Robinson, H. B. G., and Kitchin, P. C.: Actinomyces and the Dentobacterial Plaque, *J. D. Res.* **30**: 88, 1951.
5. Hodge, H. C., and Wah Leung, S.: Calculus Formation, *J. Periodont.* **21**: 211, 1950.
6. Jacobsen, N., and Kesel, R. G.: Salivary Ammonia and Its Correlation to Dental Calculus, *J. D. Res.* **29**: 364, 1950.
7. King, E. J.: Preparation of Phenolphthalein Phosphate, *J. Path. & Bact.* **55**: 311, 1947.
8. Naeslund, C.: Studies of Tartar Formation, *Acta. Path. Microbiol. Scandinav.* **3**: 637, 1926.
9. Prinz, H.: The Origin of Salivary Calculus, *D. Cosmos.* **63**: 231, 1921.
10. Rosebury, T.: The Parasitic Actinomycetes and Other Filamentous Microorganisms of the Mouth, *Bact. Rev.* **8**: 189, 1944.
11. Tenenbaum, B., and Karshan, M.: The Composition and Formation of Salivary Calculus, *J. Periodont.* **15**: 72, 1944.

RADIOPACITY OF ORAL STRUCTURES AS A BASIS FOR SELECTING OPTIMUM KILOVOLTAGE FOR INTRAORAL ROENTGENOGRAMS

ARTHUR H. WUEHRMANN* AND WILLIAM A. CURBY

Departments of Radiology and Oral Physiology, Tufts College Dental School, Boston, Mass.

CURRENT roentgenographic techniques are not capable of producing films which give suitable representation simultaneously to every oral structure on the film. Enamel is usually underpenetrated or soft tissue is overexposed. It would be valuable to obtain a single film technique which would disclose pathological changes in all the oral structures.

This goal requires a knowledge of the radiopacity range of oral structures. Measurements of this range should be carried out intraorally in order to represent oral conditions best. Such information would make it possible to predict what kilovoltage would be required in order to disclose the full range of radiopacity existing in the oral cavity. Essentially this requires a kilovoltage that will produce penetration of the maximum thickness of enamel without overexposing the minimal thickness of soft tissue.

Most clinicians have shown a preference for low kilovoltage techniques from 42 to 65 kilovolts.[1-6] Recently, Fitzgerald[7] has advised using kilovoltages higher than this and has given demonstrations of the effect of the high kilovoltage techniques on intraoral structures. The purpose of the present investigation was to try to specify the optimum kilovolts peak (kvp) more accurately and to establish this optimum by physical tests. This approach required knowledge of the radiopacity range of oral structures from enamel to soft tissue.

EXPERIMENTAL PROCEDURE

The x-ray equipment consisted of a shockproof full wave rectifier having a capacity of 200 ma. at 90 kvp or 10 ma. at 110 kvp. It was equipped with a shockproof transformer, a filament standby voltage and amperage stabilizer accurate to ± 5 per cent, a milliamperage stabilizer accurate to ± 10 per cent, and a synchronous timer with a range of 1/10 to 14 seconds accurate to 12 ± 1 impulse per 1/10 second, as checked with the spinning top. The x-ray tube was a shockproof, oil immersed, air flow, double focus type with focal spots of 1.5 and 3.8 mm. Only the 1.5 mm. focal spot was used. The output of the machine was 2.82 r per second in air at 23 cm. target skin distance, 70 kvp and 10 ma. with no extrinsic filtration. Under these conditions the half value layer (HVL) in mm. of Al was 0.8. The kilovoltage as recorded on the prereading primary voltmeter calibrated in kvp to include line drop was checked at date of installation and following completion of the investigative work. It was found to be both consistent and accurate. In a like manner the line voltage was checked. It was

Supported by contract No. V1001M-598 from Veterans Administration.

Presented at the 29th General Meeting of the International Association for Dental Research at French Lick, Indiana, March 17, 1951. (*J. D. Res.* **30**: 473, 1951.)

Received for publication July 18, 1951.

*Now at University of Alabama, School of Dentistry, Birmingham.

Fig. 1.

207 volts with no perceptible drop at 10 ma., a one volt drop at 50 ma. and a 1.5 volt drop at 100 ma. The above values were obtained at 60 kvp. A steel extension cone adjustable to lengths of 11, 15, and 18 inches with an opening of 3 inches was used. In all instances the 18 inch length and 0.5 mm. of aluminum filtration were used.

The film employed was Kodak ultra-speed film No. 4. The solutions used were of a standard formula. The films were developed at 5 minutes at 65° ± 1° F. under constant agitation. They were then washed for a minimum of 30 seconds in running water, fixed for 15 minutes, and re-washed for 20 minutes. The films were processed on hangers, agitated by hand and dried in air.

An intraoral ivory step-tablet measuring 33 mm. in length and 9 mm. in width, with 11 steps measuring 1.5 mm. in thickness except for the first step which measured 4.5 mm. was used. Ivory was selected because it was reported to be most similar to dentin in composition.[8] Film density readings were taken with a model 500 Photovolt Electronic Photometer using a No. 18 hypodermic needle as the tube for scanning. The area of coverage was 0.6 mm. in diameter.

RESULTS

The general purpose of the first phase of this study was to determine under intraoral conditions, the densities of the various dental structures in terms of known thickness of ivory. The selection of the proper milliampere second (mas) for different kilovoltages was approximated first in vitro in order to avoid overexposure of subjects. Three freshly extracted teeth were placed on ultra-speed intraoral film with the ivory step-tablet and exposed at kilovoltage peaks ranging from 110 to 35 kilovolts at different milliampere second exposures. The resultant films were evaluated visually under standard conditions and the most suitable mas for each kvp was selected. This provided a basis for intraoral exposures.

In the intraoral study, the side of the step-tablet lay on the occlusal surface of the teeth with the film in its usual position. The intraoral exposures had to be increased slightly beyond the values determined extraorally because of the density of the soft tissue of the cheek. Five exposures at kilovoltages ranging from 50 to 110 kilovolts in steps of 15 were made on six patients with ultra-speed film using the mas values selected. These were 8 mas at 110 kvp; 12.5 mas at 95 kvp; 25 mas at 80 kvp; 40 mas at 65 kvp; and 80 mas at 50 kvp. Readings were made on several areas of the developed film including enamel, dentin, pulp chambers, interproximal bone, and soft tissue. Readings were taken using the Photovolt Electronic Photometer on 4 second molars, 8 first molars, 7 premolars and adjacent bone and soft tissue. A comparison was made with the readings on the step-tablet which had been exposed simultaneously on the same film.

Table I shows the average and range of radiopacity of oral structures in terms of millimeters of ivory. Metallic restorations and enamel have radiopacity corresponding to over 19.5 mm. of ivory. Soft tissue has a radiopacity corresponding at all times to less than 4.5 mm. of ivory. The minimum radiopacity

of enamel and dentin are similar and the maximum radiopacity could not be determined. There were many more readings of enamel above 19.5 than there were in the case of dentin; hence the average difference between enamel and dentin is greater than is shown in the table. The pulp chambers show a wide range in radiopacity from 4.5 to 15 mm. of ivory because of the considerable variation in the thicknesses of the teeth and bone through which the ray penetrated. There is also a wide variation in the radiopacity of interproximal bone. This step-tablet, therefore, represents the minimum range in radiopacity of intraoral structures.

TABLE I
RADIOPACITY OF ORAL STRUCTURES IN TERMS OF IVORY THICKNESSES

TOOTH STRUCTURES	MILLIMETERS OF IVORY	
	AVERAGE	RANGE
Metallic restoration	>19.5	(>19.5)
Enamel	>18.8	(15 to >19.5)
Dentin	>18.0	(15 to >19.5)
Dento-enamel junction	18	18 to 19.5)
Cemento-enamel junction at mesial or distal margin	15	(12 to 16.5)
Pulp-chamber or canal	13	(4.5 to 15.0)
Interproximal bone	9	(6 to 15.0)
Soft tissue	<4.5-	(<4.5)

The purpose of the second phase of this study was to specify the optimum kvp necessary to give representation of all tissues on the same radiograph. It has been well established that an increase in kilovoltage results in an increase in the range of structural density which can be demonstrated on one film.[9, 10] Knowing the minimal density range of oral tissue in terms of mm. of ivory, it is possible to determine the kilovoltage which (under conditions of varying mas values) allows for complete coverage of all tissue density, and permits clear visual differentiation between adjacent steps. The ivory step-tablet was used extraorally with intraoral film to demonstrate, from a theoretical standpoint, the advantages of the higher kilovoltage technique under conditions which are not complicated by the appearance of the structures involved. The step-tablet was exposed at 6 different kilovoltages. The first exposure at each kilovoltage was made at an mas value designed to produce equal film background densities. These were 4 mas at 110 kvp; 6 mas at 95 kvp; 10 mas at 80 kvp; 15 mas at 65 kvp; 30 mas at 50 kvp; and 75 mas at 35 kvp. These are illustrated in the top line of Fig. 1. The additional exposures as seen in the vertical columns were increased 50 per cent over the exposure of the film directly above. The background density values in each horizontal row were taken to prove equality of exposure.

A slit 1 mm. wide was placed over each tablet and 10 observers were asked to count the number of varying densities which they could observe in each horizontal scale, regardless of the width of the areas. It was necessary to use the 1 mm. slit since the delineations between some steps could readily be seen and observers could use these as a cue for recognizing transitions from one step image to another. When the film being viewed was restricted to a 1 mm. width,

the line of demarcation between steps could not be observed and the density differences had to be used by the observer in estimating the number of steps seen. It is the author's impression that this viewing technique permits differences in thin steps to be distinguished more readily than would be possible in the intraoral film under the usual viewing conditions. This type of fallibility tends to detract from, rather than to support, the chief conclusion of this study.

Table II demonstrates the average number of different densities which could be observed. It will be noted that the number of perceptible density differences is most frequently greatest at 95 kvp.

TABLE II

AVERAGE NUMBER OF DIFFERENT DENSITIES OBSERVED UTILIZING VARIOUS KILOVOLTAGES WITH LOGARITHMIC INCREASES IN EXPOSURE

110	95	80	65	50	35
2.7	3.2	3.2	2.9	2.4	1.5
4.3	4.6	4.5	4.3	3.3	2.2
6.2	6.4	5.6	5.4	4.8	4.0
8.1	8.4	8.4	7.0	6.4	5.1
9.6	9.5	9.1	8.3	8.1	6.6
10.2	10.3	10.5	10.1	9.5	8.2

Further increases in mas beyond those included in this experiment will raise the number of perceptible density differences for the low kvp range. However, this procedure tends to "burn out" the less dense areas, and despite impractical and lengthy exposures, produces little additional penetration.[11] Furthermore, in the low kvp range, additional increases in mas become impracticable due to overheating of the small focal tube, the necessity for multiple exposure, increased risks of movement by the patient, and excessive skin radiation dosage.

SUMMARY

The density of oral structures in terms of mm. of ivory as seen on the average intraoral film varies beyond the limits of 19.5 and 4.5 mm. of ivory. These may properly be considered the minimal range.

If the quantity of radiation can be critically controlled it is possible to demonstrate, at kilovoltages commonly used on dental x-ray machines (approximately 65 kvp), the full scale of ivory thicknesses varying from 4.5 to 19.5 mm. Slight variations in quantity (mas) result in either overexposure of the least dense areas or underexposure of the most dense areas. If the density range exceeds the limits of 4.5 and 19.5 mm. the probability of full coverage is further reduced.

As the kilovoltage is increased with proper decreases in mas the full scale of structural density can be seen in spite of considerable variation in the quantity of radiation. This greater latitude will accommodate densities in oral structures beyond the range of 4.5 and 19.5 mm. of ivory.

On the basis of this study and clinical evidence in its support, the optimum kilovoltage for intraoral radiography appears to be 90 to 95 kvp.

REFERENCES

1. Raper, H. R.: Clinical Preventive Dentistry, Ed. 1, Rochester, New York, 1926, Ritter Dental Manufacturing Company, page 46.
2. Simpson, C. O.: Technique of Oral Radiography, Ed. 2, St. Louis, 1928, The C. V. Mosby Company, page 62.
3. Waggner, D. T.: The Principles of the Long Tube Technique, *J. Nebraska D. A.* **24**: 3, 1947.
4. Frank, Leonard: Long Distance and Low Penetration for Dental X-ray Units, *D. Digest* **44**: 114, 1938.
5. Ennis, L. M.: Dental Roentgenology, Ed. 3, Philadelphia, 1939, Lea and Febiger, page 44.
6. McCall, J. O., and Wald, S. S.: Clinical Dental Roentgenology, Ed. 2, Philadelphia, 1950, W. B. Saunders Company, pages 10 and 17.
7. Fitzgerald, G. M.: Dental Roentgenology. IV. The Voltage Factor (KVP), *J. A. D. A.* **41**: 19, 1950.
8. Mack, P. B., Brown, Jr., Walter N., and Trapp, H. D.: The Quantitative Evaluation of Bone Density, *Am. J. Roentgenology and Rad. Therap.* **61**: 808, 1949.
9. Files, Glenn W. et al.: Medical Radiographic Technique, Ed. 1, Springfield, Ill., 1949, Charles C Thomas, pages 99, 169-171.
10. Wuehrmann, A. H., and Monacelli, C. J.: Selection of Optimum Kilovoltage for Dental Radiography, Preliminary Studies, *Radiology*, **57**: 240, 1951.
11. Eastman Kodak Company, Medical Division, The Fundamentals of Radiography, Ed. 6, Rochester, New York, 1947, page 26.

DUST FACTORS INVOLVED IN THE USE OF THE AIRDENT MACHINE

MYRON J. VAN LEEUWEN, A.B., D.D.S., AND AUGUST T. ROSSANO, JR., B.S., M.S.

Harvard School of Dental Medicine and Harvard School of Public Health, Boston, Mass.

THE introduction of the Airdent machine and its increasing use by the dental profession have introduced factors not previously encountered in dental practice.

Because of the nature of the cutting agent, i.e., particles of aluminum oxide carried by a stream of carbon dioxide, the problem of recovery of the spent powder is one which demands considerable attention.

All dusts categorize themselves roughly into those capable of producing pneumoconioses—the toxic dusts, and those relatively harmless from a respiratory standpoint—the nontoxic dusts. Silica is a well-known example of a toxic dust, its inhalation producing silicosis.

Inhalation of nontoxic dusts, such as aluminum oxide, exhibits no lung pathosis but should be controlled because of "nuisance characteristics." In industry, the upper permissible limit of concentration of a toxic dust such as silica is usually considered to be 5 MPPCF,* while the upper permissible limit of a nontoxic dust is 50 MPPCF. Concentrations higher than 50 MPPCF are referred to as "poor housekeeping," and reduced concentrations are demanded in order to (1) keep the dust out of the eyes of the worker; (2) maintain good visibility during the operation; (3) protect delicate machinery from dust abrasion and (4) reduce the over-all collection of dust in the area.

Preliminary tests with the Airdent machine were carried out to demonstrate the concentration of aluminum oxide dust that accumulated in the atmosphere during cavity preparation on a patient. The over-all elapsed time for each preparation was approximately six minutes. The room approximated the average dental operating room in size, measuring 10 feet long, 8 feet wide, and 15 feet high. The abrasive dust used in the Airdent machine during these tests was aluminum oxide with the mean particle diameter of about 40 μ as determined on the microprojector. This is well above the size considered injurious from a respiratory standpoint.

Dust counts were made to determine the relative dustiness of the air in the dentist's breathing zone and in the general room during a typical operation and to determine the efficacy of modifications in the dust collection system. A Bausch and Lomb dust counter using dark field was employed.

The maximum permissible concentration for continuous exposure to 50 MPPCF already referred to is based on computation by the light field microscope counting technique. Since dark-field counting invariably yields higher counts because of improved resolution, it may be assumed that the counts obtained in these tests are well below the maximum allowable concentration.

Received for publication, Nov. 19, 1951.

*Millions of particles per cubic foot of air.

TABLE I

	DUST COUNTS (MILLIONS OF PARTICLES PER CUBIC FOOT OF AIR)			
	DENTIST'S BREATHING ZONE		MEAN ROOM CONCENTRATION	
MARCH, 1951	Average conc.	20	at beginning	2.4
	Max. conc.	30	at end	6.1
APRIL, 1951	Average conc.	6.4	at beginning	4.8
	Max. conc.	12.0	at end	5.7

It was noted that the general room dustiness increased during the operation of the Airdent machine for successive tests. We do not know whether or not the final readings represented equilibrium concentrations beyond which further increases would be unlikely. This is a function not only of the length of operation but also of the general room ventilation.

During the first test much of the spent abrasive material escaped capture by the dust removal system evidenced by the higher dust counts as well as the deposition of large quantities of dust on the patient's face. In the second test, two modifications were made. (1) The placement of the rubber dam was improved to cover a larger area of the patient's face. (2) The exhaust duct was fitted with a modified inlet hood. The results indicate that these changes were helpful in reducing the dust level in the dentist's breathing zone. In addition, very little dust was deposited on the patient's face. The results are given in Table I.

The improper application of the rubber dam is probably one of the greatest causes of accumulation of dust on the face and person of the patient. It has been found that placing the upper border of the rubber dam slightly above the tip of the nose results in greatly decreased dust accumulation on the patient's face. At the present time, rubber dam holders are being redesigned in an attempt to ascertain if improved design will result in greater efficiency of dust removal. It is felt that in order to minimize even further the nuisance properties of the uncollected dust, additional studies are indicated.

Work is progressing on newly designed inlet hoods. A further hood modification has been made but not yet tested. It is felt that additional efficiency can be brought about by refinement of the dust-collecting mechanism. Information on the particle size distribution of dust before and after use would be of great interest. In addition, the type and performance of the air cleaner used in the machine should be investigated as it is felt that a significant amount of the general room dustiness may be due to the escape of the finer dust particles in the exhausted air.

SUMMARY

On the basis of dust counts, particle size, and composition, it is felt that the normal use of the Airdent machine presents little health hazard to patient or dentist.

THE BACTERICIDAL ACTION OF CERTAIN ANTISEPTICS ON THE ORAL BACTERIA

L. W. SLANETZ AND HARRIET REYNOLDS

From the Department of Bacteriology, University of New Hampshire, Durham, N. H.

AS NEW antiseptic agents are developed, it is of interest to test their value for the control or elimination of undesirable bacteria in the mouth. The present study is a continuation of work previously reported[1, 2] and is an attempt to evaluate, by in vivo methods, the antibacterial action of Cepacol* solution, Cepacol troches, penicillin troches, and Cepacol-penicillin troches on oral bacteria. Listerine and aureomycin troches were also included for comparative purposes.

METHODS

Collection of Samples From the Mouth.—Each subject chewed a small square of paraffin approximately one-half inch in size for a 3-minute period. period. At 30-second intervals during this period, all the saliva produced was collected in a sterile 30 mm. by 100 mm. sputum bottle.

Culture Media and Plating Technique.—A blood agar medium was used for counts of general types of oral bacteria and for alpha or viridans streptococci. This medium contained 2 per cent Difco tryptose, 0.1 per cent glucose, 0.5 per cent sodium chloride, 2 per cent agar, and 5 per cent citrated sheep blood, and was adjusted to pH 7.0. For the determination of the number of oral lactobacilli, the saliva samples were plated in tomato juice agar adjusted to pH 4.8 (with lactic acid) containing 40 per cent filtered tomato juice, 1 per cent Difco peptone, 1 per cent Difco peptonized milk, and 2 per cent agar. The number of fusiform bacteria was determined by plating the samples in crystal violet potato extract agar containing 2.0 per cent Difco tryptose, 0.3 per cent Difco beef extract, 0.05 per cent cysteine HCl, 0.1 per cent glucose, 10 per cent potato extract, and 2 per cent agar at a pH of 7.4. Before pouring into plates, crystal violet was added to make a 1:15,000 concentration.

Before plating, all saliva samples were first adjusted to a total volume of 20 ml. using a phosphate buffer solution containing Tween 80 and lecithin. The samples were then shaken on a Kahn shaking machine for 10 minutes before further dilutions were prepared. The phosphate buffer diluent employed was similar to that recommended in methods for the determination of the number of bacteria on eating utensils. The stock solution contained 34 Gm. of potassium dihydrogen phosphate in 825 ml. of distilled water and 175 ml. of normal sodium hydroxide solution. The final diluent was prepared by adding 1 ml. of this solution to 800 ml. of distilled water and sterilizing in desired quantities.

To eliminate the bacteriostatic action of any Cepacol that might be present in saliva samples collected soon after medication, Tween 80 and lecithin were added to the phosphate buffer solution used to adjust the samples to the 20 ml. volume. This solution was prepared by adding 0.07 per cent lecithin

Received for publication April 7, 1951. Revised, September 14, 1951.

*Registered trademark of The Wm. S. Merrell Company, Cincinnati, Ohio.

and 0.5 per cent Tween 80 to the standard phosphate buffer diluent, boiling for 5 minutes and sterilizing. The concentration of Tween 80 and lecithin used was that recommended by Quisno, Gibby and Foter.[3] Addition of lecithin and Tween 80 in the above concentrations to the blood agar base resulted in hemolysis of the blood when the plates were incubated and thus cannot be used directly in the medium.

All samples were plated in the tryptose glucose blood agar using dilutions of 1:10,000, 1:100,000, and 1:1,000,000. Occasionally the 1:1,000,000 dilution was omitted when certain medications had been administered prior to the collection of the samples. For lacobacilli counts, dilutions of 1:100 and 1:1000 were prepared in tomato-juice agar and the plates were placed in jars in an atmosphere of 10 per cent CO_2. Dilutions of 1:50 and 1:100 were employed for the estimation of the number of fusiform bacteria and the plates incubated in jars under complete anaerobic conditions. Duplicate plates were prepared for all dilutions and all cultures were incubated at 35° C. for 48 hours.

Treatment Schedule.—The subjects used in this study ranged in age from 17 to 45 years, the majority being college students. Two methods of administering and evaluating the antiseptic action of the test agents were employed.

One method was arranged to determine the action of Cepacol solution, Cepacol troches, penicillin troches, and Cepacol-penicillin troches on the oral bacteria when used daily for a 5-day period. During the first week of the tests, each subject rinsed his mouth with a control Cepacol solution morning and night following the regular brushing of the teeth or dissolved 3 control Cepacol troches in the mouth each day, 1 about ½ hour after each meal. During the following week, each subject followed the above program but used the active or regular Cepacol solution, Cepacol troche, penicillin troche or Cepacol-penicillin troche preparations. Saliva samples were collected from each subject at 9 or 10 A.M. daily starting 1 day after the medications were first used.

The control Cepacol solution and control Cepacol troches contained all the ingredients of the active or regular preparations except the Ceepryn chloride. The penicillin troches (Lilly) contained 5000 units of crystalline-potassium penicillin G and the Cepacol-penicillin troches contained the same type and amount of penicillin plus 1:1500 Ceepryn chloride.*

A second treatment schedule was arranged to test the more immediate effect of the antiseptic agents on the oral bacteria. For this purpose, samples were collected from each subject at 10 A.M. before medication and then 1, 2, and 4 or 5 hours following medication. Thus, any changes in the number of oral bacteria could be compared to the number found in the 10 A.M. sample. The number of oral bacteria was based on total counts on blood agar and on counts for alpha streptococci, lactobacilli, and fusiform bacteria.

The treatment schedule for this series of experiments was as follows: On Tuesday and Thursday of each week the subjects rinsed their mouths for

*These test materials were supplied through the courtesy of Wm. S. Merrell Co., Cincinnati, Ohio.

1 minute with 10 ml. of the control Cepacol solution or with 10 ml. of sterile water. On Wednesday and Friday these subjects rinsed their mouths in a similar manner using the regular Cepacol solution or Listerine. Another group of subjects dissolved control troches in their mouths following the 10 A.M. sample on Tuesday and Thursday and dissolved regular Cepacol troches or Aureomycin troches in the mouth on Wednesday and Friday. The control Cepacol and regular Cepacol preparations were the same as those previously described. The Listerine was used undiluted and the aureomycin troches contained 15 mg. of aureomycin.*

Controls were prepared to determine whether any of the test substances might be present in sufficient concentration in the saliva samples to inhibit the growth of the bacteria in the blood or tomato juice agar plates. Thus, control plates were prepared by mixing suitable dilutions of saliva from a test subject with the saliva from a subject receiving no medication. Counts on such plates were then compared to those obtained when the saliva samples from these subjects were plated out separately. Counts were also made on the samples immediately after collection and 5 hours later to determine whether any bactericidal action had occurred on standing when held at 8° C.

RESULTS

When the test medications were employed twice daily over a 5-day period a very marked reduction in the numbers of oral bacteria generally occurred. A group of 8 to 15 subjects was used for testing each antiseptic. As compared to the average normal count, reductions in numbers based on the total count on blood agar averaged 94.8 to 99.5 per cent for Cepacol solution, 87.2 to 99 per cent for the Cepacol troches, 0 to 99.6 per cent for penicillin troches, and 74.6 to 99.9 per cent for the Cepacol-penicillin troches. Examples of the results obtained for some of the subjects are given in Table I.

Two subjects using penicillin troches showed essentially no reduction in the numbers of bacteria on the basis of the blood agar counts. This was due to the development in the mouth of Gram negative bacteria identified as *Aerobacter aerogenes* which were resistant to the penicillin. These organisms appeared on the third day following medication with the penicillin troches. The alpha streptococci, lactobacilli and fusiform bacteria were greatly reduced in numbers or eliminated from the mouths of these two subjects. It was observed that in all subjects taking the troches containing penicillin, the numbers of all types of bacteria were extremely low on the first and second days of the test period. On the third day, new types of organisms appeared in the samples from some of the subjects. Monilia appeared in 4 subjects. *A. aerogenes* in 2 subjects, Neisseria organisms in 1 subject, and *Serratia marcescens* in 1 subject.

Reductions in the numbers of alpha streptococci in the test subjects averaged 95 to 99.6 per cent for the Cepacol solution, 92 to 99.4 per cent for the Cepacol troches, 92.5 to 100 per cent for the penicillin troches and 94 to 100 per cent for the Cepacol-penicillin troches. The numbers of alpha streptococci in the saliva samples of the test subjects averaged 217.8 million before

*Supplied through the courtesy of Lederle Laboratories, Pearl River, New York.

TABLE I

REDUCTION IN NUMBERS OF ORAL BACTERIA FOLLOWING DAILY USE OF TEST MEDICATIONS FOR A FIVE-DAY PERIOD

MEDICATION	SUBJECT	NORMAL OR CONTROL COUNT	COUNT DURING USE OF MEDICAMENT	APPROXIMATE PER CENT REDUCTION
Mouth rinsed with 10 ml. of Cepacol for 1 minute following brushing of teeth morning and night	2	159.8	5.6	96.4
	3	206.3	6.5	96.8
	4	108.3	4.2	96.1
	5	138.0	1.3	98.9
	6	165.1	7.5	95.4
	9	180.5	0.8	99.5
3 Cepacol Troches daily, 1 approximately ½ hour after each meal	10	14.5	1.0	92.5
	11	13.5	1.7	87.2
	12	202.6	1.1	99.5
	13	108.5	1.4	98.6
	14	185.0	2.4	98.6
	17	454.0	5.2	99.0
3 Penicillin Troches (5000 units per troche) daily, 1 approximately ½ hour after each meal	Di	163.4	0.8	99.4
	Jo	73.8	127.9	0
	Rob	19.5	4.3	78.0
	Rol	80.6	10.1	87.5
	F	578.0	6.0	99.0
	L	476.0	2.1	99.6
3 Cepacol-Penicillin Troches (5000 units Penicillin per troche) daily, 1 approximately ½ hour after each meal	Re	129.4	2.1	98.3
	Sl	89.3	22.7	74.6
	Ho	110.2	9.9	90.9
	Ya	156.8	10.7	93.1
	Sa	303.5	7.1	97.7
	S	283.4	16.8	94.1

Numbers listed in millions and represent an average of the counts obtained on blood agar each day during the 5-day control and the 5-day medication period. Samples were collected daily at 9 or 10 A.M.

and 5.8 million during the use of Cepacol solution, 173 million before and 3.0 million during the use of Cepacol troches, 165.6 million before and 0.7 million during the use of penicillin troches, and 170 million before and 0.9 million during the use of Cepacol-penicillin troches. While large numbers of alpha streptococci were normally present in the mouths of all subjects tested, their elimination or reduction by the above antiseptics was greater and more consistent than for any of the other types of oral bacteria.

The reductions obtained in the numbers of oral lactobacilli were quite variable, ranging from 81.5 to 99 per cent for the Cepacol solution, 64 to 98.6 per cent for the Cepacol troches, 0 to 100 per cent for the penicillin troches, and 0 to 100 per cent for the Cepacol-penicillin troches. Examples of the results obtained for some of the subjects are given in Table II. In the majority of subjects, a definite reduction in the number of oral lactobacilli occurred following the use of the medicaments. No definite explanation for the poor results obtained for some of the subjects is available at this time.

The reductions obtained in the numbers of fusiform bacteria were also somewhat variable, ranging from 0 to 100 per cent for the penicillin troches and 51.7 to 100 per cent for the Cepacol-penicillin troches. In the majority of subjects, however, a marked reduction did occur, the numbers in the saliva samples of the test subjects averaging 60,300 before and 2,500 during the use of penicillin troches and 99,700 before and 3,200 during the use of Cepacol-penicillin troches. The bactericidal action of Cepacol solution and the Cepacol troches on the fusiform bacteria was not tested.

In considering the figures presented above for the reductions in numbers of oral bacteria following the use of the test antiseptics, it should be noted that the samples were collected 2 to 3 hours after the antiseptics were used each morning; also that the bacterial counts for each subject were based on an average of the daily counts obtained during the 5-day test period and represent the number of bacteria in the total saliva sample.

TABLE II

REDUCTION IN NUMBERS OF ORAL LACTOBACILLI FOLLOWING DAILY USE OF TEST MEDICATIONS FOR A FIVE-DAY PERIOD

MEDICATION	SUBJECT	NORMAL OR CONTROL COUNT	COUNT DURING USE OF MEDICAMENT	APPROXIMATE PER CENT REDUCTION
Mouth rinsed with 10 ml. of Cepacol for 1 minute following brushing of teeth morning and night	2	1,300.0	36.0	97.0
	3	3,130.0	481.2	84.5
	4	670.0	0.5	99.9
	5	1,317.0	243.6	81.5
	6	2,672.0	20.0	99.0
	9	1,340.0	68.0	95.0
3 Cepacol Troches daily, 1 approximately ½ hour after each meal	10	968.5	39.6	95.9
	11	240.0	164.2	64.0
	12	1,330.0	395.0	70.5
	13	990.0	223.8	77.4
	14	1,858.0	12.2	93.5
	17	1,296.0	69.7	94.6
3 Penicillin Troches (5000 units per troche) daily, 1 approximately ½ hour after each meal	Di	3,382.0	1,654.0	51.0
	Jo	16.2	0	100
	Rob	11.0	3.3	70.0
	Rol	1,031.7	2.1	99.8
	F	178.0	186.0	0
	L	356.3	113.3	68.2
3 Cepacol-Penicillin Troches (5000 units Penicillin per troche) daily, 1 approximately ½ hour after each meal	Re	1,494.0	0	100
	Sl	1,265.0	148.0	88.3
	Ho	381.4	52.5	86.2
	Ya	162.6	2.0	98.7
	Sa	96.0	171.2	0
	S	3,098.3	345.7	88.8

Numbers listed in thousands and represent an average of the counts obtained each day during the 5-day control and the 5-day medication period. Samples were collected daily at 9 or 10 A.M.

Counts were made on samples obtained from the test subjects 5 days, 3 weeks, and 4 weeks after the test antiseptics were discontinued. In general, the numbers of oral bacteria were still appreciably reduced 5 days after discontinuing the medications but they then returned to approximately the original number. However, for several subjects the numbers still appeared to be low after 3 or 4 weeks. Tests were also made to determine whether commercial tooth powder or paste might interfere with the action of the Cepacol. No inactivating action could be demonstrated when the subjects brushed their teeth with such preparations before the use of the Cepacol solution or troches. Also, no evidence was obtained which would indicate that any of the antiseptics were present in the saliva samples in sufficient concentration to inhibit or kill the bacteria present.

The following results were obtained when saliva samples were collected 2 hours after the use of control preparations (placebos), Cepacol troches, aureomycin troches, Cepacol solution, and Listerine. A group of 8 to 15 subjects was again used for testing each antiseptic.

No regular or marked decrease in numbers of oral bacteria was obtained when the control preparations (placebos) were used. In the majority of cases, the 12 A.M. samples showed a definite increase in numbers when total counts on blood agar and counts for alpha streptococci were made. The counts for lactobacilli were more variable, the 12 A.M. samples often showing a decrease even when the control preparations were used.

TABLE III

CHANGES IN THE NUMBER OF ORAL BACTERIA AFTER USE OF TEST MEDICATIONS

SUBJECT	COUNT AT 10 A.M.	COUNT 2 HR. AFTER MEDICATION	APPROX. PER CENT CHANGE	COUNT AT 10 A.M.	COUNT 2 HR. AFTER MEDICATION	APPROX. PER CENT CHANGE
	Medication: 1 Control Troche			*1 Cepacol Troche*		
H	664.2	700.5	+ 5.4	792.5	143.9	–81.9
B	545.5	328.7	– 39.8	369.5	51.8	–77.8
Re	149.5	284.8	+ 90.5	106.0	15.8	–85.1
	Medication: 1 Control Troche			*1 Aureomycin Troche*		
S	56.6	65.8	+ 16.2	150.2	84.8	–43.5
H	85.2	96.7	+ 13.4	127.2	18.7	–85.2
J	4.2	38.9	+826.0	73.0	5.8	–92.1
	Medication: Control Solution			*Cepacol Solution*		
H	399.5	387.2	– 3.0	270.5	122.5	–54.7
L	110.7	203.7	+ 84.9	89.6	24.4	–72.7
S	144.8	528.0	+264.6	279.8	45.6	–83.7
	Medication: Control Solution			*Listerine*		
B	97.5	138.2	+ 41.7	78.5	32.3	–57.5
A	202.8	751.0	+270.3	175.5	21.1	–30.9
G	104.8	82.1	– 21.6	120.0	130.5	+ 7.9

Numbers listed in millions and represent an average of 2 to 4 counts made on blood agar on different days. Samples collected at 10 A.M. before medication and at 12 A.M., 2 hours after medication.

As compared to the numbers present in the 10 A.M. samples, the reduction in numbers of bacteria based on counts on blood agar averaged 47 to 90 per cent for the Cepacol troches, 43 to 92 per cent for the aureomycin troches, 10 to 90 per cent for the Cepacol solution, and 0 to 74 per cent for the Listerine. Examples of the results obtained for some of the subjects are shown in Table III. Reductions in the number of alpha streptococci averaged 46 to 91 per cent for the Cepacol troches, 39 to 96 per cent for the aureomycin troches, 21 to 90 per cent for the Cepacol solution, and 0 to 96 per cent for the Listerine. Two subjects dissolving 2 Cepacol lozenges in the mouth showed decreases of 97 and 96 per cent in the total numbers of bacteria on the blood agar plates and 98 and 97 per cent in the numbers of alpha streptococci. All the above decreases are perhaps more significant if one considers the fact that for many of the subjects a definite increase in numbers of bacteria would have occurred following the 10 A.M. sampling period.

The reduction in numbers of bacteria obtained 1 hour and 4 or 5 hours after medication was similar to that obtained 2 hours after medication. In a few cases, the per cent decrease was somewhat less after the 4 or 5 hour periods. On certain days, some of the subjects occasionally showed no reduction in numbers of oral bacteria and such results lowered the average per cent reduction for the 5-day test period.

The numbers of oral lactobacilli varied considerably on different days and no marked bactericidal action of the test agents was evident. This was

partially due to the fact that a reduction in the numbers of oral lactobacilli apparently occurred during the 10 A.M. to 12 A.M. test period even when the control preparations were used. Further studies are being made to determine whether this is a normal or regular fluctuation in the numbers of these organisms occurring in the mouth.

No irritation was reported following the use of the test antiseptics, although a few subjects reported that the Cepacol preparations did cause some loss of taste sensitivity for a short period of time. One subject was found to be allergic to penicillin and thus could not be administered the penicillin troches.

SUMMARY

The daily use of Cepacol solution, Cepacol troches, penicillin troches or Cepacol-penicillin troches produced a marked reduction in the number of oral bacteria, including the alpha streptococci, lactobacilli, and fusiform bacteria. When penicillin was used, penicillin resistant bacteria and yeast appeared in the mouth, sometimes in large numbers. The addition of Ceepryn chloride to the penicillin (Cepacol-penicillin troche) appeared to prevent, to some extent, the development of such organisms.

Definite decreases in the numbers of oral bacteria, particularly alpha streptococci, were obtained when samples were tested 1, 2, and 4 or 5 hours after dissolving a Cepacol or Aureomycin troche in the mouth or after rinsing the mouth with 10 ml. of Cepacol solution or Listerine for 1 minute. The Cepacol troches appeared to produce greater reductions than the Cepacol solution and the Cepacol solution was more effective than the Listerine. Under the conditions of the above tests, the reductions in numbers of oral lactobacilli following the use of the test medications varied with the different subjects and the results were inconclusive. It is interesting to note that greater reductions in the oral flora occurred in the 2 subjects dissolving 2 Cepacol troches in the mouth at one time.

Considerable variation was found in the numbers of oral bacteria present in the saliva samples from certain subjects when taken on different days. This is perhaps to be expected with the uncontrollable conditions that may occur in the mouth from day to day. However, this complicates the study of oral antiseptics by in vivo methods and makes it highly desirable that an average of several tests on different days be obtained in attempting to evaluate the bactericidal action of antiseptics on the oral bacteria.

The authors wish to express their appreciation to Dr. Clara H. Bartley, Assistant Professor of Bacteriology, University of New Hampshire, for her technical assistance and advice with certain phases of this study.

REFERENCES

1. Slanetz, L. W., and Brown, E. A.: Studies on the Effect of Hydrogen Peroxide upon the Numbers of Oral Microorganisms, *J. D. Res.* **25**: 223, 1946.
2. Slanetz, L. W., and Brown, E. A.: Studies on the Numbers of Bacteria in the Mouth and Their Reduction by the Use of Oral Antiseptics, *J. D. Res.* **28**: 313, 1949.
3. Quisno, R., Gibby, I. W., and Foter, M. J.: A Neutralizing Medium for Evaluating the Germicidal Potency of the Quaternary Ammonium Salts, *A. J. Pharm.* **118**: 320, 1946.

END-POINT STUDY OF BACTERICIDAL EFFECT OF ANTIBIOTICS USED IN ENDODONTICS

LOUIS I. GROSSMAN AND CONSTANCE K. CHRISTIAN

From the Oral Medicine Department, School of Dentistry, University of Pennsylvania, Philadelphia, Pa.

ONE OF the characteristics of antibiotics is their specificity of action, i.e., they seem to have a specific destructive effect against some organisms and will have little or no effect upon others. For example, penicillin is effective against most gram-positive organisms and practically ineffective against gram-negative organisms, while streptomycin will destroy many gram-negative organisms and is helpless against most gram-positive organisms. The older antiseptics, including those used in root canal treatment, were nonspecific in their effect and generally destroyed organisms without regard to gram differentiation. Because of the selective action of antibiotics, it might be well to examine the types of organisms commonly isolated from infected root canals.

A survey of the organisms recovered in our laboratory from more than 1,000 cases of pulpless teeth (Table I) shows that 77 per cent were gram-positive (chiefly streptococci); 5 per cent were gram-negative organisms; and 16 per cent were yeasts (chiefly *Candida albicans*). The important organisms, both from the standpoint of frequency of isolation and pathogenicity, can be said to be as follows: streptococci and staphylococci as representatives of gram-positive organisms; coliform organisms as representative of the gram-negative group; and *C. albicans* as representative of yeast organisms.

In vitro tests were run to determine the end point at which three antibiotics, namely, penicillin, bacitracin streptomycin and, in addition, caprylate sodium are bactericidal against a number of varied organisms isolated from infected root canals. The organisms used in the study were: (1) alpha streptococcus (mitis); (2) beta streptococcus; (3) *S. fecalis;* (4) *S. aureus;* (5) *S. albus;* (6) *E. coli;* and (7) *C. albicans.*

MATERIALS AND METHOD

Drugs.—Dilutions of the drugs to be tested were prepared in brain heart infusion broth (Difco) as follows:

Penicillin (crystalline G) in units per ml.: 500, 300, 100, 50, 25, 12.5 6.25
Bacitracin in units per ml., 500, 400, 300, 200, 100, 50, 25, 12.5 and 6.25
Streptomycin (calcium chloride complex)—0.25 per cent, 0.1 per cent, 0.05 per cent, 0.025 per cent, and 0.012 per cent concentrations were prepared.
Caprylate sodium—33 per cent, 20 per cent, 10 per cent, 5 per cent, 0.8 per cent, 0.5 per cent, 0.3 per cent, 0.1 per cent and 0.01 per cent concentrations were tested.

Microorganisms.—Dilutions ranging from 10^{-1} to 10^{-7} were prepared from 24 hour broth cultures, as follows: alpha streptococcus; beta streptococcus,

Received for publication, Sept. 11, 1951.

TABLE I
TYPES OF ORGANISMS ISOLATED FROM ROOT CANALS

GRAM-POSITIVE			
Cocci			
Streptococcus			
alpha	289		
beta	8		
gamma	53		
enterococci	46		
Staphylococcus			
albus	157		
aureus	4		
citreus	8		
Pneumococci (?)	128		
Sarcina lutea	3		
Gaffkya tetragena	1		
Bacilli			
Bacillus subtilis	49		
Bacillus buccalis	20		
Unidentified bacilli	14		
Cornybact. pseudodiph.	5		
Lactobacilli	3	788	(77.4%)
GRAM-NEGATIVE			
Cocci			
M. catarrhalis	2		
Bacilli			
Coliform	53		
Aerobacter aerogenes	2		
Ps. aeruginosa	1	58	(5.7%)
Yeasts			
Candida albicans	171	171	(16.8%)
Total	1017	1017	(99.9%)

Streptococcus fecalis, Staphylococcus aureus, Staphylococcus albus, Escherichia coli and *Candida albicans.*

In a series of test tubes, 4.5 ml. of the required concentration of drug was pipetted. To the first tube, 0.5 ml. of microorganism was added. This was mixed thoroughly and 0.5 ml. of this mixture was pipetted to tube No. 2, etc., until the last tube was reached serially from which tube 0.5 ml. of the mixture was removed and discarded. The same procedure was followed for each of the dilutions of drug.

Inasmuch as streptomycin is inactivated by brain heart infusion broth, another medium containing peptone 1 per cent, beef extract 0.5 per cent, and sodium chloride 0.25 per cent adjusted to pH 7.8-8.0 was used in all tests with this antibiotic.

All tubes were incubated at 37° C. for 48 hours after which 0.1 ml. of each tube was transferred to flasks containing 100 ml. of brain heart infusion broth. These flasks were also incubated at 37° C. for 48 hours after which final readings were made, noting clearness or cloudiness of the culture medium. Smears were then made of all flasks showing growth and compared with the inoculant.

When *C. albicans* was being used as the test organism, all tubes were incubated at 37° C. for 48 hours. One-tenth ml. of each tube was then inoculated into 100 ml. of Sabouraud's broth and incubated at 37° C. for 48 hours, after which they were allowed to stand at room temperature for one week. Final readings were taken at the end of this time.

TABLE II
RESULTS

CAPRYLATE SODIUM (%)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
	Dilutions of Beta Streptococcus						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	#
0.8*	#	#	#	#	#	#	#
	Dilutions of Enterococcus 201 Fecalis						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.8	#	#	#	#	#	#	0
0.5*	#	#	#	#	#	#	#
	Dilutions of Alpha Streptococcus (Mitis)						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.8	0	0	0	0	0	0	0
0.5	#	#	0	0	0	0	0
0.3*	#	#	#	#	#	#	#
	Dilutions of Escherichia Coli						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.8	0	0	0	0	0	0	0
0.5*	#	#	#	#	#	#	#
	Dilutions of Staphylococcus albus 207						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.8	0	0	0	0	0	0	0
0.5*	#	#	#	#	#	#	#
	Dilutions of Candida albicans						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.8	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0
0.3	0	0	0	0	0	0	0
0.1	#	#	#	0	0	0	0
0.01	#	#	#	#	#	#	#
	Dilutions of Staphlococcus aureus						
5	0	0	0	0	0	0	0
1	#	#	#	#	0	0	0
0.8*	#	#	#	#	#	#	#
	Dilutions of Enterococcus 200 (U. of M.)						
5	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0.8	#	#	#	#	#	#	0
0.5*	#	#	#	#	#	#	#

0, negative.
#, positive.
*Positive at all concentrations tested (to 0.01%).

TABLE II—CONT'D

BACITRACIN (UNITS PER C.C.)	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
	Dilutions of Staphylococcus aureus						
500	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0
12.5	#	0	0	0	0	0	0
6.25	#	#	#	#	#	#	#
	Dilutions of Escherichia Coli						
200*	#	#	#	#	#	#	#
	Dilutions of Alpha Streptococcus (Mitis)						
500	#	#	0	–	–	–	–
400	#	#	0	0	–	–	–
300	#	#	#	0	0	0	–
200	#	#	#	#	#	0	–
100*	#	#	#	#	#	#	#
	Dilutions of Beta Streptococcus						
500	0	0	0	–	–	–	–
400	0	0	0	0	–	–	–
300	0	0	0	0	0	–	–
200	0	0	0	0	0	–	–
100	0	0	#	#	#	#	#
50*	#	#	#	#	#	#	#
	Dilutions of Enterococcus 201 Fecalis						
200	#	#	0	0	0	0	0
100	#	#	0	0	0	0	0
50	#	0	0	0	0	0	0
25*	#	#	#	#	#	#	#
	Dilutions of Staphlococcus albus						
500	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0
300	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0
12.5	#	#	#	#	#	#	0
6.25	#	#	#	#	#	#	#

0, no growth.
#, growth.
–, not run.
*Positive at all lower concentrations to 6.25 units per c.c.

TABLE III

TEST ORGANISM	PENICILLIN	BACITRACIN	STREPTOMYCIN	CAPRYLATE SODIUM
Alpha streptococcus	50 U/c.c. (10^{-7})	200 U/c.c. (10^{-6})	0.012% (10^{-3})	0.5% (10^{-3})
Beta streptococcus	50 U/c.c. (10^{-1})	100 U/c.c. (10^{-2})	0.025% (10^{-2})	1.0% (10^{-6})
S. fecalis	400 U/c.c. (10^{-3})	50 U/c.c. (10^{-2})	0.1% (10^{-4})	0.8% (10^{-7})
Staphylococcus albus	50 U/c.c. (10^{-7})	12.5 U/c.c. (10^{-7})	0.05% (10^{-2})	0.8% (10^{-7})
Staphylococcus aureus	50 U/c.c. (10^{-3})	12.5 U/c.c. (10^{-7})	0.025% (10^{-2})	1.0% (10^{-5})
Escherichia coli	400 U/c.c. (10^{-3})	200 U = No Growth	0.05% (10^{-2})	0.8% (10^{-1})
Candida albicans	----------	500 U = Growth	----------	0.1% (10^{-1})

RESULTS

The results of the tests in tabular form for caprylate sodium and for bacitracin are presented in Table II. A summary of the tests for all the drugs tested is presented in Table III.

SUMMARY

Penicillin was found to be effective against alpha streptococci in low concentration but a much higher concentration of this antibiotic was required to destroy *Streptococcus fecalis*. In exceedingly high concentration, penicillin was effective against *Escherichia coli* but was not effective against *Candida albicans* in any concentration tested.

Bacitracin was effective against staphylococci and *Streptococcus fecalis* in low concentration but was much less effective against alpha and beta streptococci. It was practically ineffective against *Escherichia coli* and completely ineffective against *Candida albicans*.

Streptomycin was effective against *E. coli*, streptococci, and staphylococci but practically ineffective against *Streptococcus fecalis* and wholly ineffective against *C. albicans*.

Caprylate sodium was effective against *Candida albicans* and against alpha streptococcus, and weakly effective against all other orgnisms tested.

Material from the same batch was used throughout the tests. For identification, the batch numbers are as follows: Penicillin G crystalline, K 526 3292 517,800 (Lilly); Bacitracin ZXD Z5057 (Upjohn); Streptomycin #2196 (Merck); Caprylate sodium R 44 (Strasenburgh). The author gratefully acknowledges receiving the antibacterial agents used in these tests from the respective manufacturers listed above.

MECHANISM OF ACTION OF SODIUM AZIDE ON THE GENUS CANDIDA

MARIE S. SLAWSON AND MARSHALL L. SNYDER

From the Department of Bacteriology, University of Oregon Dental School, Portland, Ore.

IT WAS recently reported by Diamond[1] that sodium azide incorporated in tomato-juice-peptone agar (pH 5.0) selectively inhibited oral strains of yeasts and mold contaminants frequently encountered in routine salivary lactobacillus counts. This technique was another application of a chemical previously employed to inhibit gram-negative and other bacteria to allow isolation of streptococci. The procedures for the recovery of streptococci from clinical material have been described for bovine mastitis by Bryan, Devereux, Hirschey, and Corbett,[2] for feces, urine, and exudates by Snyder and Lichstein[3, 4] and Packer,[5] and throat cultures by Pike.[6]

No difficulty was encountered in confirming the fact that a 1:10,000 concentration of sodium azide in tomato-juice-peptone agar (pH 5.0) was highly effective in the prevention of overgrowths by the yeastlike fungi. The only exception of importance was the relatively rapid loss of fungistasis in plates of tomato-juice-peptone agar held in the refrigerator. A comparison of the effectiveness of sodium azide on other members of the oral flora indicated that the mechanism of action was, as expected, to be on the iron-porphyrin proteins of these yeastlike fungi, the majority of which belong to the genus Candida.[7] Although this effect is well recognized by those interested in bacterial metabolism, such an explanation is not usually given by those reporting on the use of the chemical for clinical diagnostic procedures. On this basis, it was thought of interest to report our findings.

MATERIAL

Specimens of saliva either freshly collected or received through the mail for routine lactobacillus counts were utilized as clinical material. Pure cultures of organisms were also employed for testing purposes. These consisted of 20 strains of Candida including one strain each of four species (*C. albicans, C. kruzei, C. parakruzei,* and *C. pseudotropicalis**), 20 strains of oral lactobacilli, and 4 strains each of streptococci, staphylococci, aerobic spore-forming bacilli, and miscellaneous gram-negative types. The sodium azide was of technical grade (Eastman Kodak Co.). The medium was tomato-juice peptone agar (Hadley) adjusted to pH 5.0 or 7.0.

METHODS AND RESULTS

The evaluation of sodium azide as a selective inhibitor of oral strains of Candida was accomplished by culturing approximately 200 specimens of saliva. These specimens were diluted 1:10 in glucose broth and 0.1 ml. portions spread in duplicate over plates of tomato-juice peptone agar (pH 5.0), one series of which contained sodium azide in a 1:10,000 concentration. After four days'

*Cultures obtained from Dr. Norman S. Conant, Duke University, Durham, N. C.

incubation the plates were examined for presence of yeastlike organisms and numbers of lactobacilli counted. The results clearly confirmed Diamond in respect to the effectiveness of the azide for this purpose. Members of the genus Candida or, for that matter, of the class Eumcyetes were either completely inhibited or so restricted in growth not to interfere with the counting of the lactobacilli. This was in contrast to the presence of yeastlike organisms on 25 per cent of the control plates. Many of the latter were overgrown by these organisms. Although there were occasions when the lactobacillus count was as much as 50 per cent lower than in the controls, the majority of counts were in reasonable agreement. This reduction may be directly associated with the variation in sensitivity of strains of oral lactobacilli to sodium azide as indicated by Jay[8] and Rogosa.[9] Despite this possibility the fungistatic action of the azide permitted the successful culture of all specimens of saliva and subsequent reporting to the practitioners who are rightfully disturbed over the loss of time and effort incurred by a report "overgrown with yeasts." Thus, the advantage gained more than offset the probable error in a quantitative figure known to be subject to serious error.[10, 11] On this basis, sodium azide was incorporated as an essential ingredient of the tomato-juice peptone agar routinely used for lactobacillus counts in this laboratory.

It became apparent that the fungistasis of the azide was lost fairly rapidly in plates of this medium stored in the refrigerator. The limits for storage were established by adding varying amounts of sodium azide to tomato-juice peptone agar and pouring a large number of plates. These were held in the icebox and removed at intervals for inoculation with fresh broth cultures of yeastlike fungi. The observations are reported in Table I.

TABLE I
DURATION OF FUNGISTASIS OF SODIUM AZIDE IN TOMATO-JUICE PEPTONE AGAR HELD AT ICEBOX TEMPERATURES

RECIPROCALS OF AZIDE CONCENTRATION	NO. STRAINS	DAYS						
		1	2	3	4	5	6	7
5,000	20	0	0	0	0	0	0	10*
10,000		0	0	0	1	9	12	19
20,000		3		20				
40,000		3		20				
80,000		3		20				

*Number of strains showing growth.

Table I clearly shows the diminution of fungistasis in respect to time of storage and concentration of the chemical. There was a maximum limit of seven days for 50 per cent loss of fungistasis at the highest concentration and less than four for the lowest concentration of sodium azide in this series. This was not an unexpected finding since the chemical is recognized to be unstable. It appeared possible that its powerful reducing properties could be neutralized or offset by continued oxidation in the thin layers of the medium exposed to the atmosphere. Medium containing the azide was stored in prescription bottles, one of which was melted periodically to provide fresh plates of the tomato-juice

peptone agar. Fungistasis was retained in the bottled medium kept at room temperature for at least six weeks. It was concluded that retention of the fungistatic property of sodium azide under these conditions depended upon the exclusion of air.

At this point a question was raised concerning the possible activity of the azide against the yeastlike fungi if the normal acidity of the medium was changed. For this purpose tomato-juice peptone agar was prepared and portions adjusted to pH 5.0 and 7.0. Each batch of medium was further subdivided to allow varying concentrations of azide. Since media adjusted to pH 7.0 were to be used, the effectiveness of azide on oral bacteria which do not ordinarily grow at pH 5.0 could also be tested. All plates were inoculated by means of sterile swabs moistened with 24 hour broth cultures of the organisms employed. The plates were incubated 48 hours and examined for nature and amount of growth. The results are listed in Table II.

Analysis of Table II shows all strains of Candida, staphylococci, coliform bacteria, aerobic spore-forming bacilli and miscellaneous gram-negative types were inhibited by much lower concentrations of azide than were the streptococci and lactobacilli. Some strains of the lactobacilli even grew slightly in the presence of a 1:400 concentration of this chemical. The selective action of the azide was markedly increased at pH 5.0 for strains of Candida but this effect was less noticeable for the lactobacilli. This influence of pH upon the effectiveness of sodium azide was also reported by Keilin[12] who found that the oxygen uptake of yeast cells was completely inhibited by pH 5.2 to 6.2 by 0.001M NaN_3, 75 per cent inhibited at pH 6.7, and not inhibited at all at pH 7.5. Although it is difficult to interpolate Keilin's data obtained under the anaerobic conditions of manometric methods with the crudeness of culture media, it would appear that the increased dissociation of sodium azide in an acid environment would probably account for most of the present findings. Another feature was the recognition in these plates of the striking relationship of the fungistasis and bacteriostasis to bacterial respiration; that is, inhibition was greatest in those species classified primarily as aerobic on the basis of cytochrome content, less so for some of the facultative anaerobes and least so for those species recognized as having no iron-porphyrin proteins. Our data correlated very well with that on bacterial and yeast cytochromes tabulated in the texts of Stephenson[13] and Porter.[14] Both these authors list sodium azide as a specific poison for these enzymes.

Although direct spectroscopic evidence for blocking of cytochromes of Candida by sodium azide would be preferable, instruments were not available to perform the microscopic method described by Stephenson[13] in which she could establish the action of cyanide on the cytochromes of yeast cells. Recourse was made, therefore, to the indophenol test described by Keilin[15] as specific for cytochrome oxidase. This test is based upon the development of the purplish color of the indicator, indophenol blue, from the colorless or leuko base as the latter is oxidized by the normal respiratory process. If the iron-porphyrins

TABLE II

COMPARISON OF THE INHIBITORY ACTION OF SODIUM AZIDE ON THE GROWTH OF DIFFERENT ORGANISMS IN T J A AT pH 5.0 AND 7.0

ORGANISM	NUMBER STRAINS	pH	AZIDE CONCENTRATION (RECIPROCALS)										
			200	400	800	1,600	3,200	5,000	10,000	20,000	50,000	400,000	CONT.
CANDIDA	20	7.0	0	0	0	0	0	± (11)	++ (20)	++++	++++	++++	++++
		5.0	0	0	0	0	0	0	0	0	0	++++ (16)	++++
LACTOBACILLI	20	7.0	0	± (5)	+ (17)	++ (19)	+++ (20)	+++	+++	+++	N.D.	N.D.	+++
		5.0	0	0	± (6)	++ (20)	+++	+++	+++	+++	N.D.	N.D.	+++
STREPTOCOCCI	4	7.0	0	0	0	++ (2)	++ (3)	++ (4)	++ (4)	+++	N.D.	N.D.	+++
STAPHYLOCOCCI	4	7.0	0	0	0	0	0	± (2)	+ (4)	++	N.D.	N.D.	++++
COLIFORM	4	7.0	0	0	0	0	0	± (2)	± (3)	++ (3)	N.D.	N.D.	++++
BACILLUS	4	7.0	0	0	0	0	0	0	±	++ (4)	N.D.	N.D.	++++
MISCELLANEOUS	4	7.0	0	0	0	0	0	0	+ (4)	+++	N.D.	N.D.	++++

Degree of growth
0 No growth
± Questionable
+ Slight
++ Moderate
+++ Control
( ) No. strains showing growth
N.D. Not done

are blocked or irreversibly reduced, no color is formed. The effect of sodium azide on 13 strains of Candida including strains of the 4 species and two strains of lactobacilli was tested. All strains of Candida showed complete inhibition of cytochrome oxidase activity as measured by failure to show positive color reactions in the presence of sodium azide; these were similar to the reactions of the lactobacilli which, having no cytochromes, were negative even in the absence of the chemical. It would appear that the selective inhibition of members of the genus Candida by sodium azide was another example of its action on organisms possessing iron-porphyrin proteins and especially cytochrome oxidase.

DISCUSSION

Sodium azide as a bacterial antagonist has been employed in microbiology by two groups rather widely separated in respect to interest. The first group is comprised of clinical bacteriologists. They have utilized the selective inhibition of a broad group of bacteria by this chemical to obtain isolation of organisms otherwise difficult to recover from specimens submitted for culture. To them the end justified the means without explanation for its action. The other group has been primarily interested in bacterial metabolism rather than in diagnostic methods. To assist them in tracing the pathways of enzymatic activity, chemicals such as sodium azide which acts specifically on one or a group of enzymes have been widely employed. It is obvious that a correct explanation of its mechanism of action can come only from the studies of this group. To date, it is apparent from the literature[12-15] and our results that the major effect of sodium azide is on the iron-porphyrin components of living cells. However, Clifton and Logan[16] pointed out that azide acted on the assimilation process of synthesis (reduction) rather than on respiration (oxidation). Spiegelman, Kamen, and Sussman[17] have recently shown that, in the anaerobic fermentation of glucose by yeast cells, azide suppresses the esterification of inorganic phosphate, and Gale[18] has reported the unfavorable action of azide on external glutamic acid synthesis by a strain of *Staph. aureus*. These and other reactions of sodium azide should be expected in view of its marked toxicity, but these will be masked in ordinary cultural procedures by the great affinity of the azide for the iron-porphyrin proteins of living cells. On the other hand, as the specific points of interference are established so will it become possible for the clinical laboratory to obtain maximal utilization of this chemical as a selective inhibitor of fungi and various bacteria.

SUMMARY

The effectiveness of sodium azide as a selective inhibitor of the yeastlike organisms of the saliva has been confirmed. The limit of duration of fungistasis of a 1:10,000 concentration of sodium azide in tomato-juice peptone agar has been established as 5 days at icebox temperatures. The mechanism of action has been shown primarily to be upon the iron-porphyrin pigments, the cytochromes and cytochrome oxidases, of the genus Candida.

REFERENCES

1. Diamond, B. E., and Walstad, A.: A Selective Medium for Lactobacilli Counts From Saliva, *J. D. Res.* **29**: 8, 1950.
2. Bryan, C. S., Devereux, E. D., Hirschey, W. C., and Corbett, A. C.: The Use of Brilliant Green, Sodium Azide, and Dextrose in the Microscopic and Hotis Tests for Streptococcic Mastitis, *North Am. Veterinarian* **20**: 424, 1939.
3. Snyder, M. L., and Lichstein, H. C.: Sodium Azide as an Inhibiting Substance for Gram-Negative Bacteria, *J. Infect. Dis.* **67**: 113, 1940.
4. Lichstein, H. C., and Snyder, M. L.: The Inhibition of the Spreading Growth of Proteus and Other Bacteria to Permit the Isolation of Associated Streptococci, *J. Bact.* **42**: 653, 1941.
5. Packer, R. A.: The Use of NaN_3 and Crystal Violet in a Selective Medium for Streptococci and Erysipelothrix rhusiopathiae, *J. Bact.* **46**: 343, 1943.
6. Pike, R. M.: An Enrichment Broth for Isolating Hemolytic Streptococci From Throat Swabs, *Proc. Soc. Exper. Biol. & Med.* **57**: 186, 1944.
7. Knighton, H. T.: A Study of Monilia and Other Yeastlike Organisms Found in the Oral Cavity, *J. D. Res.* **18**: 103, 1939.
8. Jay, P.: University of Michigan. Personal communication.
9. Rogosa, M., and Mitchell, J. A.: The Indispensability of Magnesium for Lactobacillus helueticus and Its Unavailability in a Magnesium Azide Complex, *Bact. Proc.* page 130, 1950 (Paper No. 22.)
10. Permar, D., Kitchin, P. C., and Robinson, H. B. G.: Variations in Counts of Lactobacilli Made from Single Specimens of Saliva, *J. D. Res.* **26**: 475, 1946.
11. Lewis, J. B., and Hartman, A.: Method of Counting Oral Bacteria, *Oral Surg., Oral Med., & Oral Path.* **3**: 1200, 1950.
12. Keilin, D.: Action of Sodium Azide on Cellular Respiration and on Some Catalytic Oxidation Reactions, *Proc. Royal Soc.* **121**: 165, 1936.
13. Stephenson, Marjorie: Bacterial Metabolism, Ed. 3, London, New York, Toronto, 1949, pp. 19 to 27.
14. Porter, John Roger: Bacterial Chemistry and Physiology, New York, 1947, pp. 547 to 555.
15. Keilin, D.: Cytochrome and Respiratory Enzymes, *Proc. Royal Soc.* **104**: 206, 1929.
16. Clifton, C. E., and Logan, W. A.: Oxidative Assimilation of Lactic Acid by Escherichia coli, *J. Bact.* **37**: 523, 1939.
17. Spiegelman, S., Kamen, M. D., and Sussman, M.: Phosphate Metabolism and Dissociation of Anaerobic Glycolysis from Synthesis in the Presence of Sodium Azide, *Arch. Biochem.* **18**: 409, 1948.
18. Gale, E. F.: The Assimilation of Amino Acids by Bacteria. X. Action of Inhibitors on the Accumulation of Free Glutamic Acid in Staphylococcus aureus and Streptococcus fecalis, *Biochem. J.* **48**: 286, 1951.

THE CRYSTAL CHEMISTRY OF CARBONATE APATITES AND THEIR RELATIONSHIP TO THE COMPOSITION OF CALCIFIED TISSUES

DUNCAN McCONNELL

The Ohio State University, Columbus, Ohio.

VARIATIONS in the Ca:P:C ratios among analyses of teeth and bones are well known and it is generally conceded that these variations cannot be attributed to analytical error. Because it has been difficult to interpret these variations in the absence of an adequate theory for the crystal structure of carbonate apatite, certain calculations have been based upon the hypothetical compound, $3Ca_3(PO_4)_2 \cdot CaCO_3$. Inasmuch as the Ca:P:C ratios of this hypothetical compound are fixed, these calculations cannot be consistent with the analytical data, so the assumption is frequently made that more than one solid phase is present and contributes the elements Ca, P, or C.

Gruner and McConnell[11] were able to show that the mineral francolite cannot contain any component with the composition $3Ca_3(PO_4)_2 \cdot CaCO_3$, but it has since been suggested that submicroscopic particles of $CaCO_3$ can exist in francolite. This question requires further scrutiny.

To state the matter briefly, the question resolves itself into whether carbonate apatites represent (1) a single crystalline phase or (2) a heterogeneous mixture, the units of which are below the resolving power of optical microscopes. Inasmuch as dental enamel and other calcified tissues contain organic matter which merely serves to confuse the question of the composition of the "mineral phase," it seems reasonable to direct attention to the crystalline substances having related compositions. Although the complete solution of the more complex problem of calcified tissues will probably not result from detailed consideration of the crystalline minerals, it seems probable, nevertheless, that a thorough knowledge of the crystalline minerals will greatly augment an understanding of calcified animal tissues.

The question of the crystal structure of carbonate apatites is not new. Two fairly complete summaries, which emphasize chemical and mineralogical approaches, have appeared within recent years.[9, 23] Armstrong[2] has summarized those aspects dealing with calcified tissues.

In order to consider certain types of structural configurations which were virtually unknown in 1937 and in order to obtain complete reconciliation with more recent analyses, the hypothesis of Gruner and McConnell requires minor modification. Initially, however, it seems advisable to demonstrate that carbonate apatite, in the form of francolite at least, is a single phase and, therefore, is a structural variant of apatite.

Essential portions of this paper were presented orally at the "Symposium on The Crystal Chemistry of Apatites" at a meeting of the American Crystallographic Association, Washington, D. C., February 15-17, 1951.

Received for publication, June 12, 1951.

DATA ON FRANCOLITE

The mineral name, francolite, first appeared in print in 1843 but, in the absence of a complete description, the name staffelite was applied to essentially similar material from a different locality in 1866.[28] Various other mineral names have been applied to crystalline carbonate apatites, including dahllite, podolite, kurskite, and quercyite. Numerous mineralogists have recognized carbonate apatites and have applied mineral names to these substances on the presumption that they were dealing with a single crystalline phase. Within the past decade a few mineralogists have concluded that these minerals are not homogeneous substances but this was done chiefly through an examination of the structural hypothesis of Gruner and McConnell,[11] rather than an examination of the actual mineral substances. It can still be stated quite categorically that there is no direct proof that francolite is composed of two phases.

Although the diffraction patterns produced by fluorapatite and francolite are similar, x-ray spectrometer data recently obtained by the writer indicate significant differences. In both instances the samples were similar to analyzed samples from the same localities, so the chemical compositions can be considered as accurately known. Although it is beyond the scope of this discussion to furnish detailed interpretations of these data, nevertheless, it should be indicated that these measurements confirm the results of other investigators, including Altschuler.[1]

Silverman, Fuyat, and Weiser[29] have reported significant differences between the thermograms* of fluorapatite, carbonate apatites, and mixtures of fluorapatite and calcite. They were also successful in demonstrating that special solvents can be employed to distinguish between homogeneous substances (such as francolite) and heterogeneous substances (such as rock phosphates and mixtures of calcite and fluorapatite).

It is particularly significant that none of these methods of analysis has indicated the presence of calcite or aragonite in such materials as the francolite from Staffel, Germany, although these methods are quite sensitive to the presence of crystalline $CaCO_3$.

The most straightforward, single evidence of structural differences between francolite and fluorapatite is the significant difference in optical properties. The birefringence of fluorapatite is ≥ 0.004 but < 0.005, whereas the birefringence of carbonate apatites is usually between 0.006 and 0.020. Also of significance is the fact that carbonate apatites are frequently biaxial with optic angles as high as 40 degrees. Although the birefringence is greater, the mean refractive index observed for francolite is less when compared with fluorapatite, indicating thereby a discrepancy if one attempts to apply the theory of Wiener[30] and calculate the optical properties of a "mixed body" composed of fluorapatite and calcite. However, the presence of CO_3 groups within the structure of francolite will satisfactorily account for this enhanced birefringence.[23]

When considering the amount of carbon dioxide present in francolites it is important to remember that, if the second phase is $CaCO_3$, as much as 8 or

*Plots of exothermic and endothermic reactions against temperature obtained by means of differential thermal analysis.

10 per cent of it must be present. One cannot overlook the difficulties of concealing this quantity of a solid which shows pronounced tendencies to crystallize and which displays very high birefringence, within water-clear crystals of another mineral with very low birefringence. Much smaller quantities might escape attention in this way, but the author is completely unaware of any similar attempt to explain the presence of a second phase when there is no optical evidence for its presence and the amount of the second phase is supposed to exceed 5 per cent.

Were such a second phase present, it should be a simple matter to deduct its composition from the total chemical analysis, in which case the remaining constituents could be readily attributed to the structure of fluorapatite. This is by no means the case with francolite, however. If one attempts to deduct calcium oxide and carbon dioxide in equal molecular proportions from analyses of francolite, significant discrepancies appear.[10] In order to account for these discrepancies it becomes essential to assume that the five different analysts have made unusually large errors in these particular analyses—errors which significantly exceed the probable error for determinations of this sort.

We can conclude, therefore, that francolite is a homogeneous, crystalline, mineral, substances on the basis of the following data.

1. The optical properties are different from fluorapatite and cannot be accounted for by admixture of $CaCO_3$.

2. The powder diffraction patterns are different from those of fluorapatite and cannot be accounted for by admixture of $CaCO_3$.

3. The chemical compositions are different and deduction of $CaCO_3$ will not improve the allocation of other constituents to the structure of apatite.

4. The thermal data are different and cannot be accounted for by admixture of $CaCO_3$. This applies to the data obtained by Silverman, Fuyat, and Weiser,[29] but also to the fact observed by the writer: CO_2 is still present in francolite after the temperature has been raised to 1100° C. ± 25° and held at this temperature for 45 minutes.

5. The complete absence of any direct evidence for the presence of a second crystalline phase in spite of diligent effort on the part of numerous individuals to find such evidence. Indirect evidences based upon erroneous assumptions have appeared, and calculations of Ca:P ratios based upon completely inadequate determinations of phosphorus by colorimetric methods have been applied to show the nonexistence of carbonate apatites. These conclusions have been discussed and the nature and extent of the errors have been indicated specifically.[23]

THE STRUCTURE OF FRANCOLITE

The structure proposed by Gruner and McConnell[11] took into account the carbon dioxide of francolite but failed to take into account the presence of water in the structure. Four chemical analyses of francolites have appeared since 1937 and it now becomes apparent that modification of our earlier hypothesis is required in order to obtain rigorous conformity with these newer data.

The importance of $(OH)_4$ in isomorphic substitution for SiO_4 and PO_4 has recently been emphasized in connection with the structure of the clay min-

eral, montmorillonite,[21] and a garnetoid, griphite.[18] However, without resorting to these more complex examples, fundamental concepts can be clearly demonstrated with respect to the garnet-hydrogarnet series:

$Ca_3Al_2(SiO_4)_3$	Grossularite
$Ca_3Al_2(SiO_4)_2(OH)_4$	Plazolite
$Ca_3Al_2(OH)_{12}$	Ca-Al-hydrogarnet

In these isostructural compounds the Ca, Al, and oxygen positions have essentially identical coordinates, whereas the number of occupied silicon positions ranges from 3 to 0 for one-eighth of the unit cell. Clearly the additional hydrogen ions of hydrogarnet must be located in close proximity to the four oxygens in tetrahedral arrangement and can therefore be considered as $(OH)_4$ groups.

In ellestadite,[17] which has the composition $Ca_{10}(OH)_2(SiO_4)_3(SO_4)_3$, the structural interchangeability of PO_4 and SiO_4 groups has been demonstrated provided the electrical neutrality of the structure can be preserved by other types of substitution. For griphite it was necessary to assume $(OH)_4$ replacing PO_4 in the ratio 1:5. No such quantity of substitution of $(OH)_4$ for PO_4 is required in order to obtain rigorous calculation of the unit cell contents of francolite, but it should be indicated that water has been found above 300° C. for those analyses of francolite where this determination was made. All recent analyses of francolite show water above 105° C. It is concluded therefore that francolite is a hydrate in spite of the fact that all OH positions of hydroxyapatite may be filled with fluorine. (As will appear later the fluorine content may exceed that of fluorapatite.)

In considering the structure of francolite, the major consideration still relates to the manner of accommodating carbon in the lattice. A modification of the Gruner-McConnell structure was proposed by Hendricks and Hill[13]; it calls for:

$$[3(PO_4)]^{-9} \rightarrow [4(CO_3)]^{-8}$$

and results in degeneration of PO_4 tetrahedra into planar CO_3 groups. Their proposal reduces the requirements of electrons in the immediate vicinity, however, and would require decrease in electron donors to the extent of ½ Ca. Although this consequence cannot be regarded as an insurmountable difficulty, it is noteworthy that hydroxyls can be called upon to balance the electrical charges:

$$[4(PO_4)]^{-12} \rightarrow [4(CO_3)]^{-8} + [(OH)_4]^{-4}$$

Although the arrangement of CO_3 groups called for by the present structure is different from the arrangement proposed by Hendricks and Hill, the number of oxygens in close hexagonal packing remains the same. The analyses of francolites will not, however, permit any such simple stoichiometric substitutions.

Statistical substitutions of CO_3 for PO_4 in the apatite lattice appear to be of two different sorts which are schematically shown in Fig. 1. Four CO_3 groups are shown, three of which stand normal to the basal plane and are distributed symmetrically with respect to a 3-fold axis of the space group $C\ 6_3\ 1m$. The fourth CO_3 group is located on the 3-fold axis, parallel with

the basal plane. It is not, of course, presumed that these ionic groups are distributed in this manner in real crystals of francolite—nor need they be, because francolite is probably not hexagonal.*

Fig. 2 illustrates the model with respect to the transformation necessary to obtain francolite from fluorapatite. Observing the 3-fold axis of Fig. 1 from the position of the arrow, the left portion of Fig. 2 indicates the calcium atoms with coordinates ⅓ ⅔ ½ and ⅓ ⅔ 0; each is surrounded by 6 oxygens, which are portions of PO_4 groups. The right side of Fig. 2 shows these oxygens divorced from PO_4 groups to form CO_3 groups, and the calciums have been replaced by water molecules (shaded). The dimensions of water molecules are not small enough to proxy for calcium in this way ordinarily, but the contraction of the oxygens accomplished by the enhanced polarizations due to the carbon, when coupled with a slight increase in lattice dimensions, can be called upon to permit this configuration. Thus the present modified hypothesis for the structure of francolite calls for CO_3 groups replacing PO_4 groups, $(OH)_4$ groups replacing PO_4 groups, and neutral water replacing calcium ions which are adjacent to CO_3 groups parallel to the basal plane. It remains, however, to reconcile these structural concepts with the chemical composition of francolite.

THE CRYSTAL CHEMISTRY OF FRANCOLITE

It is not possible to obtain a structural picture for francolite which is as simple as would be desirable. In fact, the structure proposed by Gruner and McConnell was too simple to account for the analytical data which have subsequently appeared. Five analyses of francolite from as many localities are now available for consideration. Although there are specific differences among these analyses the most striking feature is their remarkable similarity. The localities, analysts, and literature references are as follows:

LOCALITY	ANALYST	REFERENCE
I. Staffel, Germany	R. B. Ellestad	Gruner & McConnell[11]
II. Leeds, Yorkshire	H. C. G. Vincent	Deans[7]
III. Tavistock, Devon	E. B. Sandell	Sandell, Hey & McConnell[28]
IV. Richtersvelt, So. Africa	C. J. Liebenberg	DeVilliers[8]
V. Busumbu, Uganda	Imperial Institute	Davis[5]

A sixth analysis of francolite from Milburn, New Zealand[15] is omitted because of its complexity. This analysis shows appreciable quantities of alumina and ferric oxide, and the assignment of these constituents to the lattice of apatite would merely serve to confuse the question under discussion. Chemical analyses of rock phosphates[20] are not suitable for calculations of this sort because such rocks almost universally contain more than one mineral component.

Table I contains the chemical calculations which have been applied to a composite (average) analysis of the five francolites after elimination of moisture (H_2O–105°), insoluble matter, and other reported impurities, and after recalculation of each analysis to a total of 100 per cent. The justification for this procedure lies in the fact that, if these substances are isomorphic variants of the

*Judging from the optical properties, francolite is monoclinic with $\beta \approx 120°$.

TABLE I

COMPOSITE (AVERAGE) ANALYSIS OF FIVE FRANCOLITE SAMPLES

1 OXIDES	2 WEIGHT PER CENT* (AVERAGE)	3 MOLECULAR RATIOS	4 RATIOS OF CATIONS	5 RELATIVE (+) CHARGES	6 CHARGES OF CATIONS PER UNIT CELL	7 IONS PER UNIT CELL**	
CaO	55.42	.9882	9.882			Ca	
MgO	0.22	.0055	.055	19.878	19.788	Mg	9.89
SrO	0.02	.0002	.002			Sr	
							10.00
Na_2O	0.04	.0006	.012	0.012	.012	Na	.01
H_2O	0.66	.0366	.732	0.732	0.729	H_2O	.10
						H_4	.13
CO_2	3.30	.0688	.688	2.752	2.741	C×4/3	.51
							6.00
P_2O_5	38.21	.2690	5.380	26.930	26.822	P	5.36
V_2O_5	0.05	.0003	.006				
F	4.05	.2132	—			F	
Cl	0.01						2.12
	101.70						
Less O	1.70					O	23.88
	100.00			50.304	50.092		42.00

*Each analysis was recalculated to 100.00 per cent (after deduction of the O equivalent to F) omitting H_2O below 105° C.

**The total number of ions is calculated to give the theoretical sum for the apatite structure. Actually the number of cations exceeds 16 if $3P \rightarrow 4C$ and $P \rightarrow 4H$.

same mineral, their individual compositions should be consistent with the structural hypothesis and therefore their average composition should be consistent also.

The summary data obtained from Table I are recorded in Table II, where it is noticeable that the calculations are mutually consistent to a surprising degree. The structural formula based upon Table II gives:

$$[Ca,Mg,Sr_{9.88}Na_{.01}(H_2O)_{.10}]F_{2.12}[P_{5.26}(4/3C)_{.51}(4H)_{.12}]O_{23.88}$$

which is entirely consistent with the proposed structure; all structural positions are assumed filled and the electrical charges are in balance.

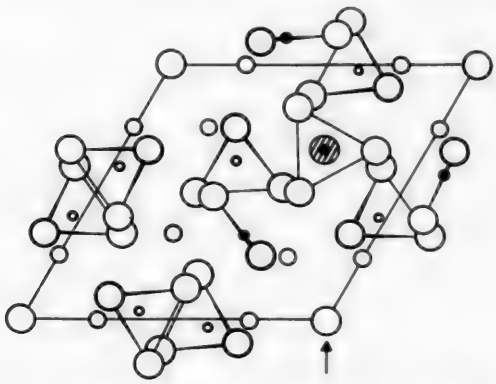

Fig. 1.—Projection on basal plane of atomic constituents of apatite wherein 3 PO_4 groups have been replaced by 4 CO_3 groups. The large circles are oxygen ions, except at vertical cell edges where they are F or OH. The large shaded circle on 3-fold axis is H_2O. Small open circles are P; small solid circles are C; and intermediate circles are Ca.

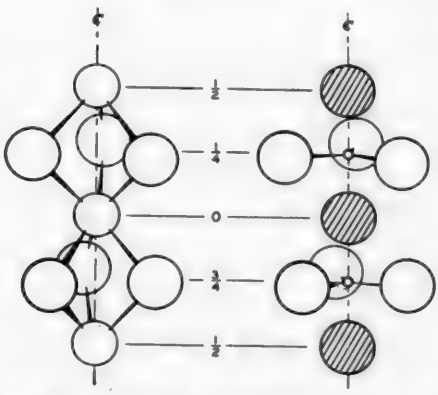

Fig. 2.—Perspective view of vertical arrangements of ions along 3-fold axis, viewed in direction of arrow (Fig. 1). Left: Ca ions surrounded by 6 oxygens in fluorapatite. Right: Carbon atoms forming CO_3 groups with oxygens, and water molecules occupying former Ca positions, as is proposed for francolite structure.

The calculations have been based upon certain well-established principles of crystal chemistry as well as the structural model described above. Only one unusual feature arises as a consequence of the calculations: the inability of the

analyses to indicate neutral water in contradistinction to hydroxyl ions. Thus the calculations cannot be balanced in terms of a knowledge of the total number of electrostatic charges nor the total number of cations, since neither is known. It therefore becomes necessary to obtain a normalizing factor which will simultaneously permit: (1) correct assignment of cations to the 16 theoretical positions of apatite, (2) preservation of appropriate ratios for their spatial equivalence, and (3) preservation of the electrical neutrality of the entire unit. The methods for making somewhat similar calculations for clay minerals have been discussed in detail.[22] Here it need only be added that the normalizing factor (Table I, column 6) can be approached by a series of trial approximations.

TABLE II.

SUMMARY COMPUTATIONS FROM COMPOSITE ANALYSIS

ELECTRICAL EQUIVALENCE			SPATIAL EQUIVALENCE		
Ca,Mg,Sr	2 × 9.89	19.79	Ca,Mg,Sr	9.89	9.89
Na	1 × 0.01	0.01	Na	0.01	0.01
H_2O	0 × 0.10	—	H_2O	0.10	0.10
P,V	5 × 5.36	26.80			10.00
C	4 × 0.68	2.72			
H	1 × 0.52	0.52	P,V	5.36	5.36
		+49.83	C	¾ × 0.68	0.51
			H	¼ × 0.52	0.13
O	2 × 23.88	47.76			6.00
F	1 × 2.12	2.12			
		−49.88	O	23.88	23.88
			F	2.12	2.12
					26.00

THE CRYSTAL CHEMISTRY OF CALCIFIED TISSUES

Although the above deliberations probably will not materially aid the biochemist in writing a structural formula for the apatite phase of calcified animal tissues, it can probably be assumed that he will not be able to do so under any circumstances until he is thoroughly familiar with all of the crystal chemical concepts pertinent thereto. Surely it can be presumed that calculations of the carbonate apatite composition of enamel, which are based on the formula $Ca_9(PO_4)_6 \cdot CaCO_3$, cannot possibly be correct because there is no such compound, and this fact has been repeatedly demonstrated. The biochemist has an immeasureably more difficult task because of the critical nature of the water content of the carbonate apatites and their intimate association with other volatile constituents in calcified tissues. Thus it may prove quite impossible to remove the organic matter of dentin without significantly altering the composition of the mineral phase. Subsequent calculation of several mineral phases, without any direct evidence of their presence, can only serve to confuse the matter further.

Thus in light of the extreme complexity of the crystal chemistry of apatite it is necessary to attack the simpler problems first. Much of this work has already been done. Klement[16] has synthesized $Na_6Ca_4(SO_4)_6F_2$ and found it to have the structure of apatite. Likewise Minguzzi[24] was able to synthesize pleochroic green crystals of chlorapatites containing both trivalent and hexavalent chromium. Pieruccini[25] has suggested that aluminum can substitute for both

calcium and phosphorus in the structure of apatite. The latter type of substitution is not particularly surprising when considered in light of Yoder's[31] demonstration of complete replacement of silicon by aluminum in synthetic yttrium garnetoid, although substitution of AlO_4 for SiO_4 was previously supposed to be very limited in orthosilicates. It must be concluded, therefore, that the structure of apatite is an exceedingly tolerant one with respect to modification by isomorphic substitution and, in accordance with the structural model described here, there are these three possible modes of entry of water into the lattice:

(1) hydroxyls which substitute for fluorine to yield hydroxyapatite, (2) hydroxyls which substitute statistically for PO_4 and ideally represent $(OH)_4$ groups, and (3) neutral water which substitutes for calcium under certain special conditions. In addition the CO_3 groups can be relegated to two types: (1) those which lie parallel to the basal plane and thereby significantly contribute to the enhanced birefringes and (2) those in other orientations.

Deakins and Burt[6] have made a pertinent observation based upon their study of the various different developmental stages of the enamel of pigs: ". . .these elements [Ca, P, and C] are deposited as a complex compound having a fixed composition." This important conclusion indicates that the dental enamel (of pigs at least) is composed essentially of one inorganic compound. In light of the well-established fact that this compound always produces the powder diffraction pattern of an apatite, it can be concluded that dental enamel is essentially a carbonate apatite with low fluorine content, essentially similar in composition to the mineral dahllite.

That heating of bone to 900° C. or above can produce crystalline compounds other than apatites is not particularly surprising.[14] In fact, reactions within the solid phase would seem probable under these circumstances, particularly if these dahllites are hydrates, as has been demonstrated for francolite. Therefore, it is concluded that the wide range of Ca:P ratios obtained for calcified tissues are not inconsistent with the conclusion that these substances are carbonate apatites. Indeed, no straightforward method has ever demonstrated that, in their natural condition, these substances are anything other than carbonate apatite, except possibly for certain pathological conditions of considerable rarity.[27] Treatment of bone and tooth substance with various reagents has yielded such minerals as brushite,[4] $CaHPO_4 \cdot 2H_2O$. Another related phosphate mineral, whitlockite β-$Ca_3(PO_4)_2$, is known to be stable at ordinary temperatures and has been recognized as a constituent of certain urinary calculi.[26] However, the x-ray diffraction patterns of these substances are quite different from those of apatite and presence of diffraction lines attributable to these compounds have not been reported for teeth or normal bones.

In 1930 Cape and Kitchin[3] concluded ". . . that enamel is not an insoluble colloid but is composed of a mass of crystals, of appreciable microscopic size, whose optical properties are measurable and definite." This important discovery of 21 years ago is common knowledge today. Likewise, it is now common knowledge that these crystals produce an apatite-like x-ray diffraction pattern. What appears to be obscure from the viewpoint of the biochemist or dental research worker is the fact that these compounds have analogues among

minerals and, although these minerals are completely crystalline, homogeneous phases, no such simple formula as $Ca_9(PO_4)_6 \cdot CaCO_3$ can be used to describe their compositions. In light of the isomorphic variations which occur among certain other mineral series, the structural hypothesis here proposed for francolite does not seem unreasonable. Furthermore, the exceedingly nice manner in which the composite analysis can be calculated to fit the structural hypothesis cannot be regarded as a fortuitous circumstance in light of the exacting requirements of calculations of this sort.

In the absence of adequate data on the chemical properties of the mineral, dahllite, no attempt has been made to reconcile its composition with the crystal structure of francolite. The only recent analysis of dahllite is one reported by McConnell[19] and in this case certain significant discrepancies in the water content appeared when the water content was determined for several ranges of temperature by two different analysts. In view of the critical nature of the water content, it seems inappropriate to consider this mineral in detail on the basis of this single analysis. The fluorine content, however, was accurately determined by Armstrong and found to be 0.19 per cent. Likewise the CO_2 content was accurately determined as 4.46 per cent. This mineral, with its low fluorine content, is apparently just as real in its existence as is francolite. Here again, the presence of no second crystalline carbonate phase has ever been demonstrated.

Inasmuch as the earlier conclusions of Gruner, McConnell and Armstrong[12] were predicated upon the somewhat inadequate hypothesis of Gruner and McConnell, it follows that these conclusions are subject to minor modification. This is primarily the consequence of the fact that hydroxyls were presumed to enter the structure only in substitution for fluorine of fluorapatite. In light of present considerations this concept is no longer tenable.

SUMMARY

It is concluded that rigorous calculation of structural formulas for dental enamel will not be possible until analytical procedures have been devised for determining three different types of water (including hydroxyls). The higher organic content of dentin and bone will greatly increase the analytical difficulties when attempting to obtain quantitative information on the types of water present. Differential thermal methods may aid in the solution of these perplexing problems.

Inasmuch as the atomic ratios Ca:P:C are not constant for calcified animal tissues, and likewise are not constant for the carbonate-apatite mineral, francolite, calculations based upon the hypothetical compound $3Ca_3(PO_4)_2 \cdot CaCO_3$ are necessarily anomalous. But the variability of the Ca:P:C ratios does not constitute a valid reason for assuming that more than one crystalline phase is present, and no direct evidence for the existence for a second phase exists.

On the basis of the proposed structural hypothesis for francolite, it is possible to obtain crystal chemical calculations which account for the isomorphic substitution of carbon in the lattice of apatite. Presumably, similar conditions obtain for dental enamel and probably also for dentin and bone.

REFERENCES

1. Altschuler, Z. S.: Oral Presentation at the Washington Meeting of the Am. Crystallogr. Assoc., February, 1951.
2. Armstrong, W. D.: *Annual Rev. Biochem.* **11**: 441, 1942.
3. Cape, A. T., and Kitchin, P. C.: *J. A. D. A.* **17**: 193, 1930.
4. Dallemagne, M. J., and Mélon, J.: *Bull. soc. chim. biol.* **27**: 597, 1945.
5. Davis, K. A.: *Econ. Geol.* **42**: 137, 1947.
6. Deakins, M., and Burt, R. L.: *J. Biol. Chem.* **156**: 77, 1944.
7. Deans, T.: *Mineral. Mag.* **25**: 135, 1938.
8. DeVilliers, J. E.: *Am. J. Sc.* **240**: 443, 1942.
9. Eisenberger, S., Lehrman, A., and Turner, W. D.: Chem. Rev. **26**: 257, 1940.
10. Geiger, T.: *Schweiz. Min. Petr. Mitt.* **30**: 161, 1950.
11. Gruner, J. W., and McConnell, D.: *Z. Krist.* **97A**: 208, 1937.
12. Gruner, J. W., McConnell, D., and Armstrong, W. D.: *J. Biol. Chem.* **121**: 771, 1937.
13. Hendricks, S. B., and Hill, W. L.: *Science* **96**: 255, 1942.
14. Hirschman, A., Sobel, A. E., Kramer, B., and Fankuchen, I.: *J. Biol. Chem.* **171**: 285, 1947.
15. Hutton, C. O., and Seelye, F. T.: *Trans. Royal Soc. New Zealand* **72**: 191, 1942.
16. Klement, R.: *Naturwiss.* **33**: 568, 1939.
17. McConnell, D.: *Am. Mineral.* **22**: 977, 1937.
18. McConnell, D.: *Am. Mineral.* **27**: 452, 1942.
19. McConnell, D.: *Am. J. Sc.* **236**: 296, 1938.
20. McConnell, D.: *J. Geol.* **58**: 16, 1950.
21. McConnell, D.: *Am. Mineral.* **35**: 166, 1950.
22. McConnell, D.: *Clay Minerals Bull.* (London) **1**: 179, 1951.
23. McConnell, D., and Gruner, J. W.: *Am. Mineral.* **25**: 157, 1940.
24. Minguzzi, C.: *Per. Mineral.* (Rome) **12**: 343, 1941.
25. Pieruccini, R.: *Atti Soc. Toscana Sc. Nat.*, Mem. 54, 12 pp. 1947.
26. Prien, E. L., and Frondel, C.: *J. Urol.* **57**: 949, 1947.
27. Reed, C. I., Reed, B. P., and Gardner, W. V.: *Endocrinology* **38**: 238, 1946.
28. Sandell, E. B., Hey, M. H., and McConnell, D.: *Mineral Mag.* **25**: 395, 1939.
29. Silverman, S. R., Fuyat, R. K., and Weiser, J. D.: Oral Presentation the Washington Meeting of the Am. Crystallogr. Assoc., February, 1951.
30. Wiener, O.: *Abh. Math.-phys. Kl. sächs. Ges. Wiss.* **32**: 507, 1912.
31. Yoder, H. S., Jr.: *Am. Mineral.*, **36**: 519, 1951.

CALCIUM EXCHANGE IN ENAMEL AND DENTIN AS SHOWN BY CALCIUM^{45}

ELIZABETH E. UNDERWOOD, M.S., AND HAROLD C. HODGE, Ph.D.

The Division of Pharmacology and Toxicology, Department of Radiation Biology, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

THE USE of radioisotopes has permitted the study of the mechanisms, the pathways, and especially the rates of mineral metabolism to be investigated. Until recently little work had been done on calcium^{45} since the radioisotope, Ca^{45}, is somewhat difficult to measure and the activities of the samples available have not been very high. Recently a number of papers have appeared; all the workers are in agreement that substantial portions of a dose of radiocalcium rapidly reach the calcified in vivo tissues.[1] The rapidity of the process is surprising; it is certain that the Ca^{45} found in the bones and teeth in short time periods has not been deposited there solely as a result of the growth of new bone or tooth tissue. The experiments in vitro reported herewith provide evidence that the rapid phase of deposition of Ca^{45} is an exchange reaction in which the surfaces of the crystals of hydroxylapatite play an important role.

EXPERIMENTAL

Samples were prepared of fresh and glycol-ashed dentin, and of dried enamel. The tooth tissues were prepared from dried human teeth from which carious material and fillings were carefully removed before pulverizing to 60 to 100 mesh size. Dentin and enamel were separated by centrifugal flotation, and the purity of each sample was established by particle count. A portion of the dentin was glycol ashed.

Radiocalcium, Ca^{45}, half-life 180 days, was obtained from the Clinton Laboratories, Oak Ridge, Tenn. The solutions were prepared by dissolving 125 mg. calcium carbonate in the calculated amount of 3 N hydrochloric acid and heating to drive off carbon dioxide. This solution was diluted to about 800 ml. with distilled water, and the pH adjusted to approximately 7.5 using 0.001 N ammonium hydroxide. Any argon present as a radio-contaminant[2] was removed by bubbling air vigorously through the solution for 15 minutes prior to making the volume 1 L. with distilled water.

Nonradioactive calcium solutions were prepared from iceland spar by solution in hydrochloric acid. Three calcium concentrations were used: specifically, 1.25×10^{-2}, $\times 10^{-3}$, $\times 10^{-4}$ M., respectively. The solutions used in the adsorption experiments were analyzed for calcium before and after exposure of the hard tissues by the method of Salomon, Gabrio, and Smith.[3]

Selected solutions and some of the hard tissue samples were analyzed for phosphorus by the method of Fisk and Subbarow.[4]

The pH was determined using the Beckman pH meter and a glass electrode calibrated against a standard pH 7 phosphate buffer at 25° C.

Received for publication, June 27, 1951.

TABLE I

GLYCOL-ASHED DENTIN

CONCENTRATION OF SOLUTION	1.25×10^{-2} M. Ca		1.25×10^{-3} M. Ca	
TIME OF EXPOSURE	% ON DENTIN	% EXCHANGED	% ON DENTIN	% EXCHANGED
1 hour	3.5	2.55		
2 hours	10.3	8.11	20.6	2.15
4 hours	13.9	11.6		
8 hours	14.9	12.4	64.3	14.9
16 hours	14.5	12.2	70.2	19.5
1 day	14.6	12.1	71.1	20.5
2 days	15.6	12.9	69.0	18.4
3 days	15.7	12.8	67.5	19.8
4 days	15.2	12.5	70.1	19.3
5 days			69.2	18.4
6 days	16.9	13.7	69.7	19.1
7 days	17.1	14.0	70.5	19.9
8 days			69.2	18.8
9 days	17.7	14.6	70.9	20.1
10 days	16.5	13.6		
12 days	16.6	14.0		

TABLE II

FRESH DENTIN

CONCENTRATION OF SOLUTION	1.25×10^{-2} M. Ca		1.25×10^{-3} M. Ca	
TIME OF EXPOSURE	% ON DENTIN	% EXCHANGE	% ON DENTIN	% EXCHANGE
8 hours			9.0	0.87
16 hours	2.79	2.58	9.9	0.90
1 day	2.40	2.07	13.4	1.39
2 days	3.14	2.74	19.0	2.16
3 days	3.53	3.20	22.3	2.57
4 days	3.51	3.18		
5 days	4.17	3.88	21.7	2.35
6 days	4.85	4.49	24.7	3.06
7 days	6.55	6.05	28.3	3.93
8 days	9.74	9.48	27.7	3.62
9 days	9.96	9.82	30.1	4.14
10 days	13.5	13.7	29.6	6.22
12 days	17.3	17.7	43.2	7.72
14 days	18.2	19.7	43.8	7.51
16 days	19.5	21.6	46.1	8.10
18 days	20.4	23.0	49.3	8.66
20 days	21.5	24.6	50.9	9.86
24 days	21.4	24.5		
25 days			53.5	11.4
26 days	22.1	25.5		
28 days	21.6	24.5		
30 days	21.5	24.7	57.8	13.6

Radioactivity determinations for calcium were made using a mica window counter of window thickness 3–4 mg. per square centimeter. The tube was purchased from the Radiation Counter Laboratories, Chicago. Two ml. aliquots of solutions were placed in a stainless steel cup and counted directly if the activity exceeded three times the background count of 12 counts per minute.

Samples of very low activity were evaporated to dryness under an infrared lamp. This procedure greatly enhanced (10-fold) the counting efficiency; the standard error was much higher in counts of dried samples but the percentage standard error was much lower.[1]

The adsorption procedure was carried out as follows: 50 mg. samples of dentin or enamel were placed in conical 50 ml. centrifuge tubes to which were added 25 ml. samples of the calcium solution of the desired concentration. The tubes were stoppered with rubber stoppers and were placed in a shaking machine for the short-time studies and in a revolving holder for the long-time studies. In both cases the agitation was carried on in a room maintained at 40° C. At the end of the designated time periods, the samples were centrifuged, the solutions decanted, the tubes drained and the bone was dissolved without washing in 3 ml. of 3 N nitric acid.

RESULTS

The time curves of adsorption from 10^{-3} M. calcium chloride are given in Fig. 1. The data for the glycol-ashed bone taken from the paper of Falkenheim, Neuman, and Hodge[5] show evidence of a rapid adsorption of Ca^{45} to an extent of approximately 60 per cent of that in the original solution. The glycol-ashed dentin (Table I) adsorbs Ca^{45} with equal rapidity and to an equal or slightly

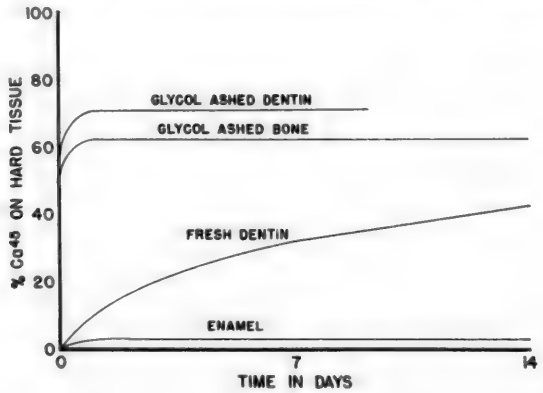

Fig. 1.—Adsorption of Ca^{45} from 1.25×10^{-3} M. $CaCl_2$ solution.

greater extent, i.e., about 70 per cent. Fresh dentin (Table II) adsorbs much more slowly and in 14 days had taken up only 40 per cent of the Ca^{45} from the solution. Enamel (Table III) adsorbs only about 2 per cent and shows little tendency to an increase in time within 14 days.

To follow the course of the adsorption and to compare the results from experiment to experiment, it has been convenient to use a term, the specific activity ratio defined by the following equation:

$$\text{specific activity ratio} = \left[\frac{\text{mg. Ca}}{\text{c.p.m. } Ca^{45}}\right]_{\text{solution}} \left[\frac{\text{c.p.m. } Ca^{45}}{\text{mg. Ca}}\right]_{\text{bone}} \times 100$$

In Fig. 2, the specific activity ratios (SAR) are plotted for fresh and for glycol-ashed dentin when exposed to 10^{-2} and 10^{-3} M. calcium chloride solutions. Glycol-ashed dentin adsorbed more calcium^{45} from the more dilute solution; in both cases the SAR's exhibit a phase of very rapid increase in which most of the adsorption occurs followed by a prolonged period of little or no increase. The SAR's for fresh dentin are practically superimposed for approximately the first week, regardless of concentration, and at this time the SAR's are much smaller than for the glycol ash. After the first week, however, the SAR's at 10^{-3} M. continue a nearly linear increase for a period of at least a month to a value of about 15. In contrast, the SAR's at 10^{-2} M. rises rapidly and in about three weeks reach a value of about 25, after which there is little increase.

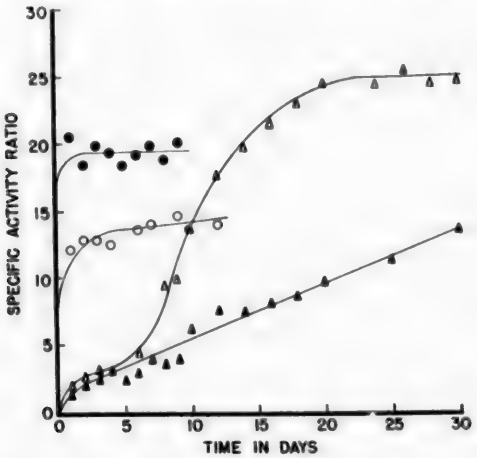

Fig. 2.—Specific activity ratios for fresh and for glycol-ashed dentin at CaCl$_2$ solutions of approximately 10^{-2} and 10^{-3} M.

● 10^{-3} glycol-ashed dentin
△ 10^{-2} fresh dentin
○ 10^{-2} glycol-ashed dentin
▲ $^{-3}$ fresh dentin

DISCUSSION

Exchange vs. Deposition.—In bone, the adsorption of Ca^{45} has been shown to be a reversible exchange reaction[1] as was previously demonstrated for P^{32}.[5] It is easy to justify the exchange concept. In Fig. 1, for example, 60 per cent of the Ca^{45} is taken from the solution by the solid bone powder in a relatively few hours. The solution in question contained 1.27 mg. calcium initially. If 60 per cent of all of the calcium had been deposited on the bone powder, the solution finally should have contained 1.27 – 0.76, or about 0.5 mg. Instead by analysis, the calcium content averaged 1.48 mg.[5] Since Ca^{45} deposition had not been accompanied by a proportionate net deposition of nonradioactive calcium, it is assumed that Ca^{45} in solution had exchanged with Ca^{40} on the bone. The reversibility of this reaction also was established by desorption experiments that demonstrated a similarity in the ultimate distribution of Ca^{45} between bone and solution, regardless of whether the Ca^{45} was initially present in the bone or in the solution.

TABLE III

ENAMEL

CONCENTRATION OF SOLUTION	10^{-2} M. Ca		10^{-3} M. Ca	
TIME OF EXPOSURE	% Ca^{45} ON E.	% EXCHANGED	% Ca^{45} ON E.	% EXCHANGED
1 hour	0.45	0.33		
2 hours	0.51	0.37		
4 hours	0.49	0.40	0.00	0
16 hours	0.78	0.63	2.7	1.6
1 day	0.99	0.74		
2 days	0.81	0.69	0.7	0.6
3 days	1.09	0.95		
4 days	0.90	0.73		
5 days	0.85	0.74	2.3	2.10
6 days	0.95	0.83	2.6	2.22
7 days	1.03	0.87		
8 days	0.95	0.79		
10 days			2.2	2.39
12 days			2.0	2.50
14 days			2.3	2.09

The concept of exchange applies equally well to the adsorption of Ca^{45} by dentin and enamel. Glycol-ashed dentin, in fact, follows a pattern of adsorption nearly identical to that of glycol-ashed bone. In contrast, enamel adsorbs Ca^{45} so slowly that it is difficult to be sure whether the same kind of reaction is involved. Assuming that something comparable to an equilibrium condition is established, the Ca^{45} is distributed between dentin or enamel and solution in such a way that all of the solution calcium and a part of the dentin or enamel calcium are involved. At equilibrium, the SAR has special significance; it has been called the exchangeable fraction.[1] Bone has a large exchangeable fraction; nearly 25 per cent of the calcium of glycol-ashed bone apparently can exchange with solution calcium. In the case of glycol-ashed dentin, the exchangeable fraction amounts to about 20 per cent after 9 days, and in the case of enamel the exchangeable fraction is of the order of 2 per cent (10^{-3} molar solutions).

Rates of Reaction.—The rates of adsorption of Ca^{45} by glycol-ashed bone and by dentin are compared with that by enamel samples in Fig. 3. In each case, the adsorption approximates a simple exponential function. The values (log Δ) in Fig. 3 plotted against the adsorption time are the logarithms of the differences between the percentage of Ca^{45} adsorbed on the bone at a given time as compared to the maximal percentages in each case. The reaction rates may be characterized by the half-time constants. The data in Table IV show that the adsorption is half completed by glycol-ashed bone or dentin in a matter of hours whereas days are required for fresh bone or dentin to reach the same sta-

TABLE IV

RATE CONSTANTS (HALF-TIME VALUES)

TREATMENT	BONE		DENTIN		ENAMEL	
(Calcium Conc.)	10^{-2} M.	10^{-3} M.	10^{-2} M.	10^{-3} M.	10^{-2} M.	10^{-3} M.
Glycol Ash	16 hrs.	2 hrs.	6 hrs.	8 hrs.		
Fresh	4 days	8 days	12 days	20 days	10 hrs.	32 hrs.

tistical point. The more rapid achievement of the half-time value in the case of enamel is an indication that the adsorption may be largely limited to the surfaces of the powder particles.

When the corresponding values for the adsorption of P^{32} on dried dentin are plotted against time, smooth curves (Fig. 4) are obtained in each case. It should be noted that the concentrations here are 2×10^{-1}, $\times 10^{-3}$, and 10^{-5} M. While the basis for this difference in rate characteristics is obscure, one fact is plain, namely, that the mechanism for the adsorption of calcium is probably not identical with the mechanism of adsorption of phosphorus. A similar statement may be made concerning the adsorption curves for P^{32} on enamel at the same concentrations (Fig. 5) although the data at 10^{-5} M. are almost linear. Falkenheim, Underwood, and Hodge[1] pointed out that the adsorption of calcium on bone is an exponential process, whereas the adsorption of P^{32} was hyperbolic. A hyperbolic course frequently is described by the P^{32} exchange for bone and enamel, but this did not apply in the case of dentin.

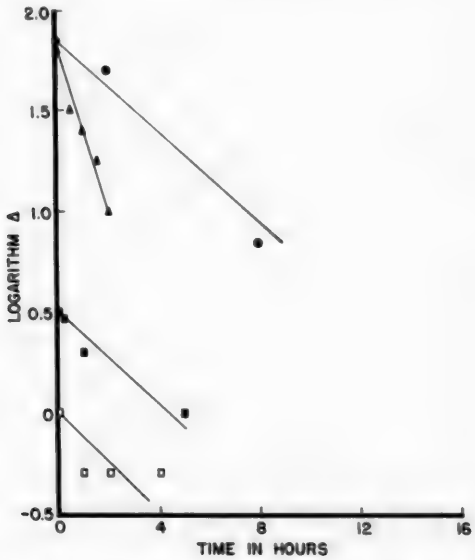

Fig. 3.—Rates of Ca^{45} exchange with glycol-ashed bone and dentin and fresh enamel.
● 10^{-3} glycol-ashed dentin
▲ 10^{-3} glycol-ashed bone
■ 10^{-3} enamel
□ 10^{-2} enamel

Exchange vs. Recrystallization.—Neuman and Mulryan[6] have described the slow phase of P^{32} fixation by bone as a recrystallization process. Falkenheim, Underwood, and Hodge[1] have shown that the slow phase of Ca^{45} exchange with bone is more or less comparable with the P^{32} exchange. The data in Tables I and II show that fresh dentin exhibits a markedly different time course of Ca^{45} exchange than does glycol-ashed dentin. In Fig. 6, the plotted data show that at 10^{-3} M. calcium concentration, fresh dentin follows an exponential course with what appear to be randomly distributed variations. In the same figure,

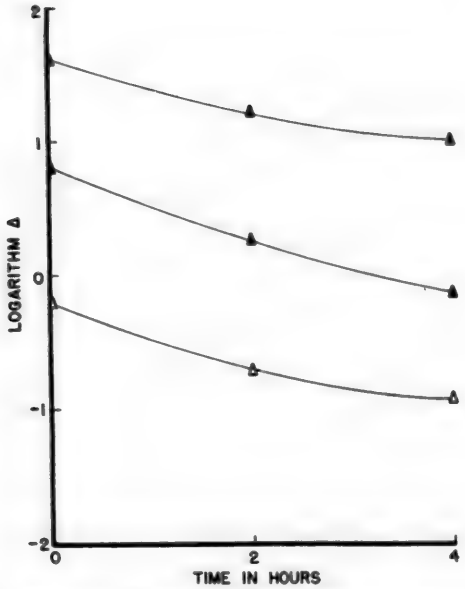

Fig. 4.—Rates of exchange of P^{32} with dried dentin at concentrations of approximately 10^{-1}, 10^{-2}, and 10^{-3} M. Phosphate (Data of Falkenheim[3]).

▲ 10^{-3} M dried dentin
△ 10^{-1} M dried dentin

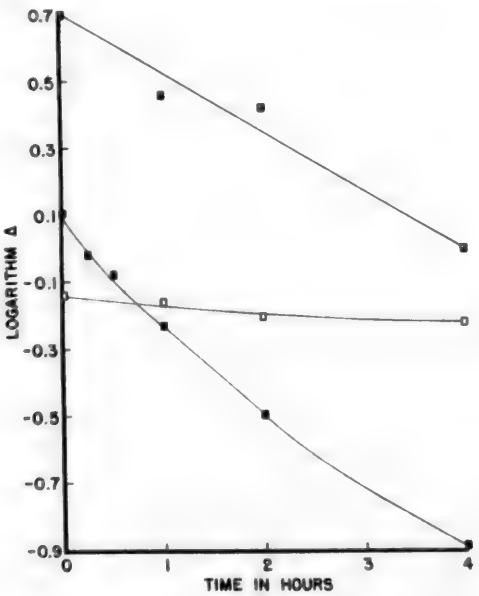

Fig. 5.—Rates of exchange of P^{32} with enamel at approximately 10^{-1}, 10^{-2}, and 10^{-3} M.

◢ 10^{-2} M enamel
□ 10^{-1} M enamel
■ 10^{-3} M enamel

the data at 10^{-2} M. describe a peculiar course: during the first week, the data fall along a straight line of small slope, after which for a period of approximately a week there is another linear tendency, but of much greater slope; the data recorded for the longest exposure times (14 to 16 days) appear to fall on a straight line through the initial time point. This raises a question as to whether the aberration in the early part of the experiment may be due to some cause unrelated to the exchange phenomena. It might be suggested, for example, that the concentration of calcium in the solution produces an effect on the protein of the dentin matrix in such a way that the penetration of Ca^{45} is hindered during the first 10 days.

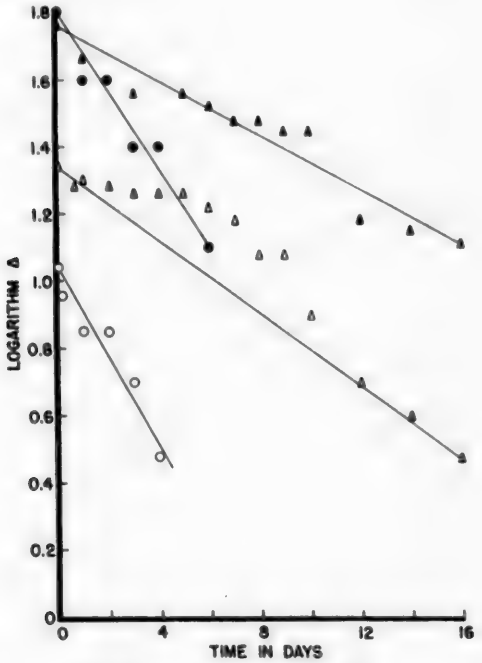

Fig. 6.—Rates of exchange of Ca^{45} with fresh dentin and fresh bone at concentrations of approximately 1^{-2} and 10^{-3} M.
▲ 10^{-3} M fresh dentin
△ 10^{-2} M fresh dentin
● 10^{-3} M fresh bone
○ 10^{-2} M fresh bone

Neuman and Mulryan[6] and Falkenheim, Underwood, and Hodge[1] found it necessary to use two lines to describe the rates of calcium and phosphorus exchange in fresh bone (observation period of a month). It is debatable whether two lines need to be applied to data on fresh dentin shown in Fig. 6 (period of 16 days). This by no means rules out the possibility that the appearance of Ca^{45} in the powdered dentin is the result of a combination of processes one of which is an exchange with calcium ions on the crystal surfaces and another is the recrystallization process involving the solution of small crystals and the growth of larger ones.

Crystal Surface.—The importance of the crystal surfaces in mineral metabolism already has been mentioned. The surfaces referred to are not the surfaces of the powder particles in the case of bone and dentin, although it is entirely possible that the enamel adsorption is largely limited by powder particle size. Hendricks and Hill[7] have recently shown that the adsorbing surface of powdered enamel increases from 2.1 m^2 per gram for 60-120 mesh powder to 6.8 m^2 per gram for particles that pass through a 325 mesh screen. The crystals responsible for the exchange with Ca^{45} probably are the very minute hydroxylapatite crystals laid in the matrix substance of dentin or, in the case of enamel, constituting most of the body of the enamel rod. In Table V, the average crystal length as determined by x-ray diffraction studies by

TABLE V

TISSUE	CRYSTAL SIZE	DENSITIES	SPECIFIC SURFACES	% EXCHANGE Ca 1.25×10^{-3} M (AT END OF RAPID PHASES)	% EXCHANGE PO_4 1.55×10^{-3} M
SOURCE	Bale[8] (cm.)	Manly, Hodge, Ange[9]	Wood[10] (sq. m/Gm.)		Falkenheim, Neuman, Hodge[1]
BONE	10^{-6}	2.0	100.0	13.5	12.0
DENTIN	10^{-6}	2.2	2.4	14.9	1.8
ENAMEL	10^{-4}	2.9	1.8	1.0	0.25

Bale,[8] an estimate of the average density of each tissue,[9] the specific surfaces as determined by Wood,[10] and the exchangeable fractions of calcium and phosphorus are given. Enamel has a considerably larger exchangeable calcium fraction than was found for phosphorus. As has been pointed out before, the exchangeable fraction of bone calcium and of phosphorus are entirely comparable. In the case of dentin, the exchangeable calcium fraction is as great or greater than that of bone, whereas the exchangeable phosphorus fraction was previously determined to be only ⅙ that of bone. Since the Ca^{45} exchange was measured on glycol-ashed dentin and the P^{32} exchange on dried dentin (organic matter still present), the difference may be ascribed tentatively to the presence of organic matter. The near equality of the surfaces indicated from the calcium study would be expected on the basis of Bale's x-ray data[8] in which the average crystal length (10^{-6} cm.) was of the same order of magnitude as in bone. It is possible from values for the exchangeable fraction to make a rough approximation of the size of the enamel crystals. Assuming the crystals are cubic, the area of N particles is $6\ N\ L^2$ (where L is the length of the side), and for 3 Gm. of enamel, this is 3×1.8 m^2 or approximately 6×10^4 sq. cm. The volume would be $N\ L^3$ or 1 c.c. Solving these equations gives an approximate "length" of the hydroxylapatite crystals of enamel of 1×10^{-4} cm. This is in reasonable agreement with the size reported by Bale. It is an interesting fact, perhaps only coincidence, that the average diameter of enamel rods is about 4 microns and this is 4×10^{-4} cm. To the x-ray beam, the principal repeating distance may be the calcified enamel rod. To the Ca^{45} ion in solution, the surface available for adsorption exchange may also be that more or less formed by the histological unit of the enamel rod.

SUMMARY

1. The adsorption of Ca^{45} by powdered dentin and enamel from aqueous calcium solutions (1.25×10^{-2}, $\times 10^{-3}$, and $\times 10^{-4}$ M.) has been measured in vitro.

2. On glycol-ashed dentin, the rapid phase of calcium adsorption is an exchange reaction in which as much as $\frac{1}{5}$ of the total calcium atoms of the dentin may ultimately take part. In fresh dentin, a slower but even more extensive exchange may occur. On enamel, only 2 per cent of the calcium atoms appear to exchange.

3. The adsorption probably takes place on the surfaces of the ultramicroextensive exchange may occur. On enamel, only 2 per cent of the calcium atoms scopic hydroxylapatite crystals. These crystals are much larger in enamel (10^{-4} cm. in length) than in dentin (10^{-6} cm.).

REFERENCES

1. Falkenheim. M., Underwood, E. E., and Hodge, H. C.: *J. Biol. Chem.* **188**: 805, 1951.
2. Isotopes Branch, United States Atomic Energy Commission, Catalogue and Price list, Radioisotopes, Oak Ridge, March, September (1947).
3. Salomon, K., Gabrio, B. W., and Smith, G. F.: *Arch. Biochem.* **11**: 433, 1946.
4. Fiske, C. H., and Subbarow, Y.: *J. Biol. Chem.* **66**: 375, 1925.
5. Falkenheim, M., Neuman, W. F., and Hodge, H. C.: *J. Biol. Chem.* **169**: 713, 1947.
6. Neuman, W. F., and Mulryan, B. J.: *J. Biol. Chem.* **185**: 705, 1950.
7. Hendricks, S. B., and Hill, W. L.: *Proc. Nat. Acad. Sc.* **36**: 731, 1950.
8. Bale, W. F., Hodge, H. C., and Warren, S. L.: *Am. J. Roentgenology* **32**: 369, 1934.
9. Manly, R. S., Hodge, H. C., and Ange, L. E.: *J. D. Res.* **18**: 203, 1939.
10. Wood, N. V., Jr.: *Science* **105**: 1272, 1947.

ELECTRON MICROSCOPY OF MATURE HUMAN ENAMEL

DAVID B. SCOTT,* MARIE J. USSING,** REIDAR F. SOGNNAES,*** AND RALPH W. G. WYCKOFF****

National Institutes of Health, Bethesda 14, Md., and Harvard School of Dental Medicine, Boston, Mass.

EXAMINATIONS of mature human enamel under the electron microscope have in the past been conducted by means of replicas of the surfaces of ground sections.[1-6] Replica techniques were early introduced into electron microscopy to study very dense materials which could not be obtained in sufficiently thin form of direct observation. They reveal only surface structure, but whereas with teeth, etching is possible, they can also give many indications of internal structure. Previous replica studies have demonstrated the general appearance of enamel at higher magnification,[1-6] the presence of prism sheaths,[1-6] and the existence of a fine fibrous network within and between the prisms.[4, 6] Some observations have been interpreted to indicate that the organic framework of the enamel decreases in quantity with advancing age,[6] and that it contains spiral configurations.[4] Little has been seen of the crystalline matter in the enamel, though a few micrographs have been published which have been thought to show crystal outlines.[4]

The development of thin sectioning techniques for the direct observation of soft tissues under the electron microscope has provided a new approach to the study of enamel. Although fully mineralized enamel cannot be sectioned by these methods, demineralized enamel can be handled with considerable success. Such sections are especially instructive, and contribute much to a better understanding of the replica studies.

The present report describes the appearance of mature human enamel as observed in replicas and in thin sections. It is concerned only with the major structures that make up the enamel, that is, the prisms, interprismatic substance, and the organic framework associated with them.

METHODS

Collodion replicas of the surfaces of ground tooth sections were prepared by methods already described.[3] The sections, about 1 mm. thick, were cut from extracted teeth on a machine which employs a thin rubber-bonded silicon carbide cutting wheel revolving at high speed under running water. Longitudinal, transverse, and sagittal sections were prepared, as well as sections cut parallel to the tooth surface and containing enamel alone. One face of a section was polished to a high finish on metallographic emery papers. The polished surface was next etched by immersion in acid. The principal acid used was

Received for publication, Aug. 3, 1951.

*National Institute of Dental Research.

**National Institute of Dental Research; Present address: National Dental College of Denmark, Copenhagen.

***Harvard School of Dental Medicine.

****National Institute of Arthritis and Metabolic Disease.

hydrochloric, in concentrations ranging from 0.01 N to 1.0 N. Several other acids were used to a lesser extent, including 0.01 to 1.0 N nitric, and 0.2 N to 0.8 N citric and lactic acids. Exposures ranged from 1 to 10 seconds, and were terminated by washing in distilled water. After etching, the surface was thoroughly dried in a gentle stream of compressed air, then covered with a few drops of collodion, U.S.P., diluted 1:6 with ether, and allowed to dry in air. In order to remove the tooth substance, the section was floated, collodion side up, on the surface of 18 per cent HCl for at least 24 hours. The replica was then transferred for further cleaning, to the surface of a 1 per cent pepsin (1:10,000) solution, pH 1.8, and incubated at 37° C. for 8 hours. It was next washed by two transfers to the surface of fresh distilled water. Electron microscope specimen screens were placed on the replica, which was face down on the water, and the screens and replica together were picked up with absorbent paper. The replica was then shadowed with chromium and examined with an RCA type EMU electron microscope. More than 500 replicas were made in this way, about 1500 specimen screens have been examined, and over 2000 micrographs taken.

Thin sections of demineralized enamel were prepared employing essentially the methods of dehydration, embedding, and cutting described by Newman, Borysko, and Swerdlow.[7] The initial material consisted of cross sections of teeth, about a millimeter thick. These were demineralized in collodion bags according to a method described by Sognnaes.[8] A section was placed in the bottom of a collodion bag, which was then suspended on a string in a 250 ml. flask containing about 200 ml. of 5 per cent trichloracetic or 5 per cent phosphoric acid. The flask was stoppered and placed in a cold room at 5° C. for one to two weeks. The big was then transferred to distilled water for several hours, and the specimen thus washed free from acid.

The demineralized tooth section was next placed in the center of a water-filled Petri dish by carefully holding the bag so that its bottom rested gently on the bottom of the dish, and cutting the bag off with a scissors at a level a few millimeters above the specimen. Accessible portions of the remains of the bag were then gently removed from around the section. Water was next pipetted slowly from the Petri dish to a level just above the top of the specimen. Sufficient 95 per cent alcohol was added to the remaining water to bring the final concentration to approximately 40 per cent. Hourly changes of alcohol were made, the concentration being raised about 20 per cent each time, ending with 3 changes of absolute alcohol. During this alcohol dehydration the remains of the collodion bag dissolved completely. Approximately half of the alcohol was then removed from the Petri dish and replaced with an inhibitor-free mixture of N-butyl methacrylate and methyl methacrylate in a ratio of 9:1. One hour later this liquid was removed to the level of the specimen and replaced with fresh monomer mixture. Four similar changes were then made at hourly intervals. The final infiltrating medium, made up of the same monomer mixture to which enough of the catalyst, 2,4-dichloro-benzoyl peroxide, had been added to provide a concentration of 1 per cent, was then introduced in the same manner. After several hours of infiltration the specimen was ready for embedding.

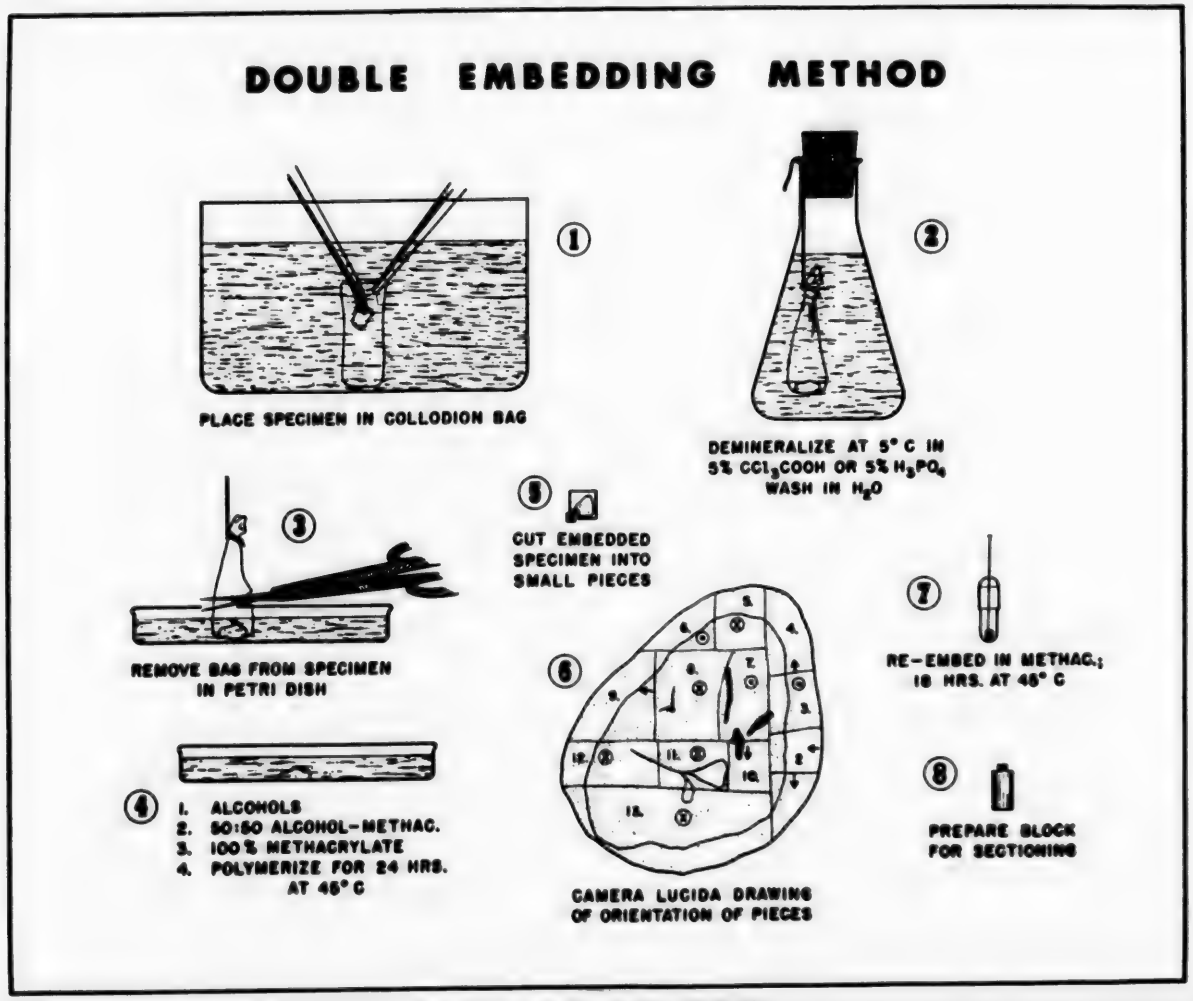

Fig. 1.—Double Embedding Method.

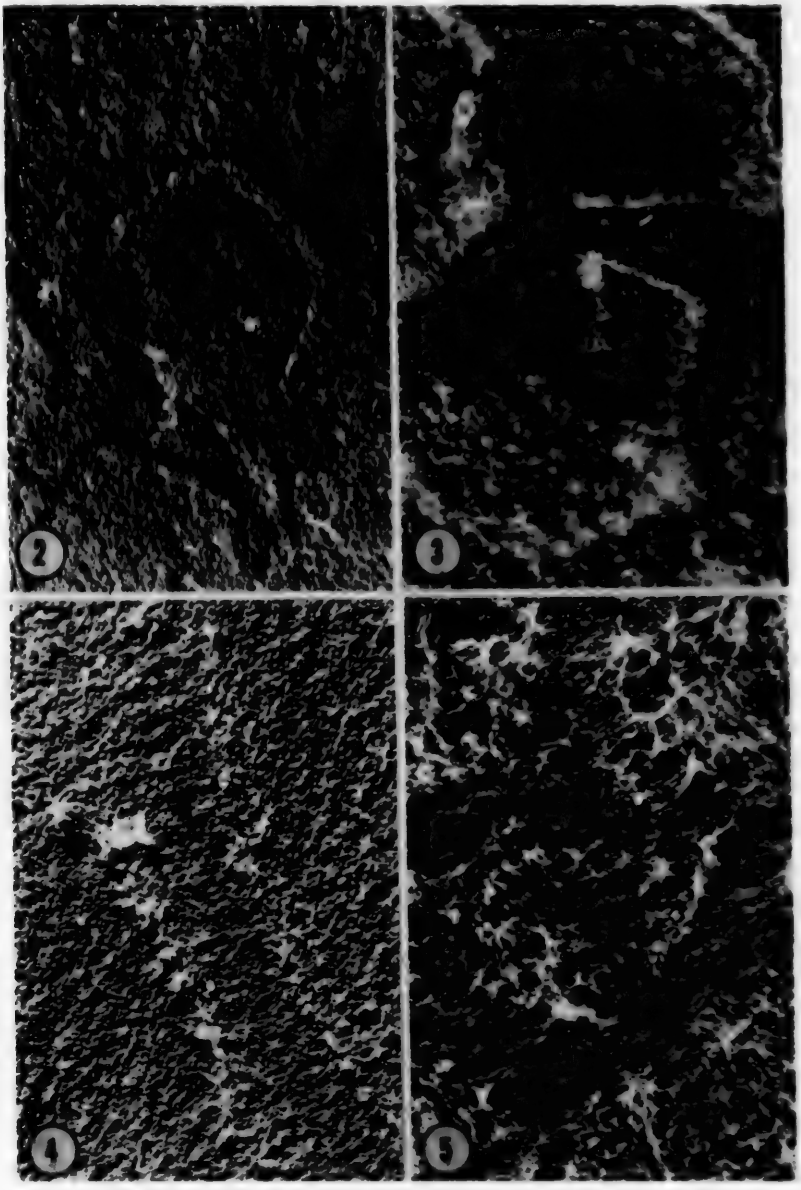

Fig. 2.—Replica of enamel, showing indistinct prism sheaths and traces of fibrillar network. (Cross-section, etched 3 sec. with 0.1 N HCl) ×7,200.

Fig. 3.—Replica of enamel showing prominent prism sheaths and granular texture of prismatic and interprismatic substance. (Cross-section, etched 5 sec. with 0.1 N HCl) ×7,200.

Fig. 4.—Replica of enamel, showing two indistinct prism sheaths, and parallel fibrils (A) in interprismatic substance. (Cross-section, etched 12 sec. with 0.1 N HCl) ×11,300.

Fig. 5.—Replica of enamel, showing prominent submicroscopic fibrils and no prism sheaths. (Cross-section, etched 5 sec. with 0.1 N HCl) ×7,200.

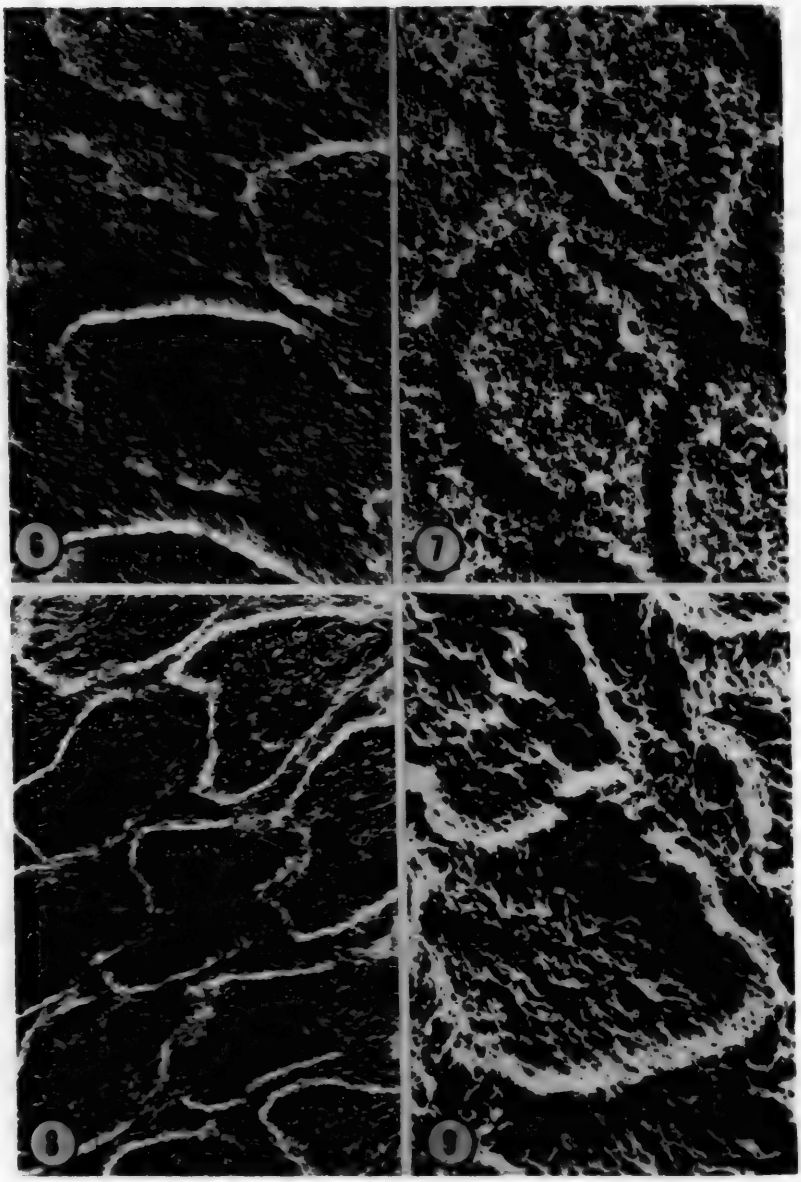

Fig. 6.—Replica of enamel, showing pronounced prism sheaths and traces of fibrillar network. (Cross-section, etched 5 sec. with 0.1 N HCl) ×7,200.

Fig. 7.—Replica of enamel, showing large quantity of submicroscopic fibrils. (Cross-section, etched 5 sec. with 0.1 N HCl) ×7,200.

Fig. 8.—Cross-section of demineralized enamel, showing prism sheaths and submicroscopic fibrillar network. ×5,800.

Fig. 9.—Cross-section of demineralized enamel, showing at high magnification one prism and part of two adjoining prisms. ×9,600.

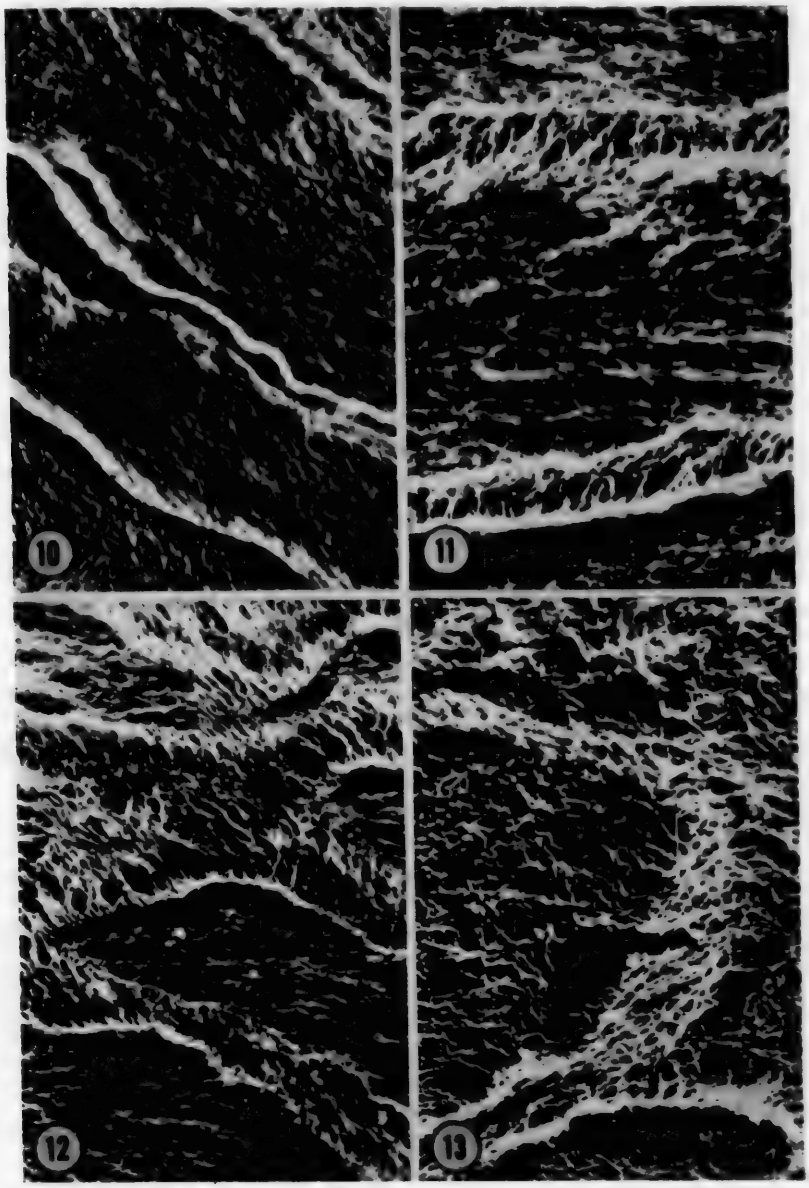

Fig. 10.—Longitudinal section of demineralized enamel, showing prism sheaths and submicroscopic fibrillar network. ×5,800.

Fig. 11.—Longitudinal section of demineralized enamel, showing at high magnification the interprismatic fibrils extending crosswise between prism sheaths. ×9,600.

Fig. 12.—Oblique section of demineralized enamel, made up principally of interprismatic substance, and showing crosswise course of fibrils between prism sheaths. ×5,800.

Fig. 13.—Exceptionally thin section of demineralized enamel, showing an obliquely cut prism sheath which appears in itself to be fibrillar. ×9,600.

According to the embedding method recommended by Newman, Borysko and Swerdlow,[7] a small piece of tissue, not over 1 cu. mm. in size, is placed in the bottom of a gelatin capsule (size 00) filled with methacrylate-catalyst mixture. The plastic is then polymerized in an oven at 45° C. during a period of 6 to 8 hours. While it was possible to dissect out the small pieces of demineralized enamel required by this method of embedding, subsequent electron micrographs showed that the dissection and handling had produced evident damage. Furthermore, this procedure made it difficult during embedding to control the orientation of the material to be sectioned, and, as a consequence, the relation of the subsequent sections to the morphological outlines of the original tooth could not be clearly visualized. These limitations were avoided by the use of a double embedding method, diagrammatically outlined in Fig. 1. In this procedure, after several hours of final infiltration, the Petri dish, containing the specimen and methacrylate-catalyst mixture, is placed in the oven at 45° C. for 16 hours to cause polymerization. The Petri dish is broken away from the hardened plastic, and the central portion of the plastic, containing the specimen, is cut out with a razor blade. The embedded specimen is then placed under a dissecting microscope, to which a camera lucida is attached, and small cubes of embedded enamel of proper size for sectioning are cut out with a razor blade. Before the specimen is dissected a camera lucida drawing is made of its outlines, and as each cube of enamel is removed its location and outline are recorded, as well as the direction from which sectioning is to take place. This provides a means for later determining the orientation of the sections in relation to the site on the tooth at which the enamel was originally located. Each cube is then placed on the end of an insect mounting pin which has previously been pushed through the center of the top half of a gelatin capsule. The cubes are so oriented that the side destined to be sectioned faces the bottom end of the capsule. The lower halves of the capsules are then filled with the same methacrylate-catalyst mixture as previously used, and the specimen-bearing top halves set in place. After polymerization at 45° C. for about 8 hours, the end of the block containing the specimen is reduced in the usual manner to the size required for sectioning.

A Spencer rotary microtome equipped with the thermal expansion adapter described by Newman, Borysko, and Swerdlow,[7] and glass knives, described by Latta and Hartman,[9] have been used for sectioning. The sections were transferred, as cut, by means of a camel hair brush from the knife to the surface of a 50:50 dioxane-water mixture, and then to a water surface. They were picked up from the water on formvar-coated electron microscope screens, allowed to dry, and the plastic was removed by immersion for one hour in amyl alcohol, followed by a similar period in amyl acetate. The mounted sections were finally shadowed with palladium at a 4:1 angle to enhance their stability under the electron beam, and to improve contrast. Approximately 1000 micrographs were made from about 500 sections cut from 40 blocks.

THE STRUCTURE OF ETCHED ENAMEL AS OBSERVED IN REPLICAS

Very few replicas were observed which were entirely free from remnants of the organic portion of the enamel. The principal clue to this organic material

on the replicas was the constant finding that the organic framework of the enamel appeared as a positive structure, elevated above the surface of the replica. In the case of a true replica this would be taken to indicate that the organic framework had been etched to a greater degree than the surrounding inorganic matter, and thus was more acid-soluble. In view of what is known about demineralization of enamel and the subsequent presence of its insoluble organic components, such a conclusion is obviously not warranted. It appeared, therefore, that the positive structures were elements of the organic tissue actually present on the replicas. That this was the case was readily demonstrable, for on some replicas the residue was so plentiful that the preparations had become too thick for electron microscopy. In such instances the organic matter was visible even to the naked eye, as a grayish white layer on the surface of the collodion. While the replicas were floating on a water surface this substance could be teased away with a glass needle. If the replicas were picked up on glass slides it could be stained and looked at under the optical microscope.

The quantity of organic material remaining on the replicas generally increased with the amount of etching to which the enamel had previously been subjected. A partial explanation for this is to be found in the physical attachment of the organic material to the collodion. This was manifest in the continuity between the collodion membrane and the overlying residuum, and in the obviously proper orientation of the organic framework with respect to the outlines of the enamel prisms, as observed under the electron microscope. Evidently, in these preparations, the superficial organic layer of the ground section, exposed through etching, was embedded in the collodion during replication, in the same manner as are the particles picked up from the surfaces of solids during the deliberate preparation of "pseudoreplicas."[10]

The typical appearance of replicas of etched enamel under the electron microscope is illustrated in Figs. 2 to 7. The principal structures distinguished in almost all preparations were the enamel prisms, interprismatic substance, prism sheaths, and a submicroscopic fibrillar network both between and within the prisms. The visibility and clarity of these structures depended largely on the amount of organic matter remaining on the replicas. There has been no evidence that etching with different acids brings out different details. It was repeatedly observed, as in previous studies,[5] that the response to acid varied considerably in different areas from the same tooth, but not according to any special pattern.

Enamel prisms had much the same outlines in the replicas as when observed directly in ground sections under the optical microscope. In most replicas the prisms were delimited by narrow peripheral structures, which undoubtedly were the prism sheaths. They usually were well-defined (Figs. 3 and 6), though occasionally indistinct (Figs. 2 and 4), and even imperceptible (Fig. 5). Some of the factors responsible for these variations in appearance have undoubtedly been the angle at which the prisms were sectioned, the degree to which the ground sections were etched, and the amount of organic matter remaining on the finished replicas.

A submicroscopic fibrillar network was almost universally present within and between enamel prisms. In some regions only faint traces were noted (Figs.

2 and 6), in others it was more pronounced (Fig. 4), and in still others it was very prominent (Figs. 5 and 7). Fig. 3 is typical of the few areas in which it could not be seen. Conclusions have not yet been reached about the orientation of the fibrils within the prisms, but those in the interprismatic substance seemed to lie more or less parallel and to run crosswise between prisms (Figs. 4, 5, and 6). Spiral organic configurations within the prisms, as previously reported,[14] were not seen.

Thus far no details have been recognized on the replicas which might be interpreted as outlining crystals of the inorganic component of the enamel. Where the organic fibrils were not so numerous as to interfere with observation of the underlying replicas, the texture of both the prismatic and interprismatic substance had only a diffuse granularity (Fig. 3).

THE STRUCTURE OF DEMINERALIZED ENAMEL AS OBSERVED IN THIN SECTIONS

Thin sections of demineralized enamel obviously can show only its organic component. As previously observed in the replicas, this appeared to have two constituents—the prism sheaths and a submicroscopic fibrillar network extending throughout both the prismatic and interprismatic regions. The striking resemblance between replicas and sections of enamel is brought out by comparisons of Figs. 6 to 9.

Well-defined prism sheaths could be distinguished in all sections (Figs. 8 to 13). They usually were dense and devoid of fine detail (Figs. 8 to 12), but exceptionally thin sections of obliquely cut sheaths gave indications that the sheaths themselves may be made up of closely packed submicroscopic fibrils (Fig. 13).

The interprismatic material appeared as an unoriented spongelike network (Figs. 8 to 12). Longitudinal sections have brought out both the parallelism of the fibrils in the interprismatic regions, and their oblique or right-angled course between prism sheaths (Figs. 10 and 11), as previously observed in the replicas. Some sections consisted principally of interprismatic substance, and the particularly good picture they afforded of the arrangement of the interprismatic fibrils confirmed what was observed in the longitudinal sections (Fig. 12).

DISCUSSION

Early studies by optical microscopy, utilizing ordinary and polarized light[11, 12] and by x-ray diffraction,[13] have been interpreted to indicate that no organic matter exists between the crystallites in mature enamel. With the refinement of methods for demineralization it has been shown by optical microscopy that mature enamel does contain an organic framework appearing to consist mainly of prism sheaths.[14] The present electron micrographs demonstrate, further, that a submicroscopic fibrillar network permeates the enamel prisms and the interprismatic subtance. The previous failure to discern these minute organic components is to be attributed to the fact that the fibrils are only a few hundred A° wide, and are well below the resolving power of the optical microscope. It is thus easy to understand why only the relatively thick prism sheaths have previously been seen, and the prism cores have usually

appeared empty.[14] It is harder to explain the solid prism cores which have been reported in studies of demineralized fully mature enamel.[15] Possibly, there might be sufficient light scattering from the well-preserved submicroscopic network of a fairly thick section to make the prism cores appear partially opaque, or the enamel may not have been completely demineralized.

In the present replica studies the inorganic crystals in fully mature enamel have not been seen, and it is probable that the so-called crystal outlines pointed out earlier in micrographs made from replicas[4] were due to the fibrillar organic network. It is conceivable that the dimensions of the network may indicate the maximum size attained by elements of the inorganic component, but whether these elements are single crystals or crystalline aggregates is not determined. In the electron micrographs of sectioned demineralized enamel now at hand the fibrils which make up the organic framework have ranged between 20 to 100 mμ in thickness, averaging 50 mμ. The openings in the meshwork, which presumably contained the inorganic fraction of the enamel, averaged 200 mμ in diameter, with a range between 150 and 300 mμ. The accuracy of these tentative measurements can not be predicted until more is known about the action on the tissue of the various chemical agents used, and about distortions which may possibly result from cutting and other manipulations. Notwithstanding, even such admittedly crude estimates make it appear doubtful that the enamel crystallites could reach a size of about one micron, as estimated by Moller and Trommel.[16] On the other hand, crystals in the order of 270 mμ., a mean size calculated by Bale, Hodge, and Warren[17] and corroborated by Thewlis,[18] could be accommodated within the openings in the meshwork as observed.

SUMMARY

Methods are described for making replicas of the etched surfaces of ground sections through enamel, and thin sections of demineralized enamel. The principal organic structures observed under the electron microscope in both types of preparation have been the prism sheaths and a submicroscopic fibrillar network within and between the prisms. The inorganic portion of the enamel, as represented in replicas, has shown a uniformly granular texture, and thus far it has not been possible to demonstrate the outlines of the individual crystals.

REFERENCES

1. Gerould, C. H.: Ultra Microstructures of the Human Tooth as Revealed by the Electron Microscope, *J. D. Res.* **23**: 239, 1944.
2. Gerould, C. H.: Electron Microscope Study of the Mechanism of Fluorine Deposition in the Teeth, *J. D. Res.* **24**: 223, 1945.
3. Scott, D. B., and Wyckoff, R. W. G.: Electron Microscopy of Tooth Structure by the Shadowed Collodion Replica Method, *Pub. Health Rep.* **62**: 1513, 1947.
4. Syrrist, A.: En orientering i elektronmikroskopien med noen resultater fra histologiske undersøkelser av emalje og dentin, *Odont. Tidsk.* **2**: 79, 1949.
5. Scott, D. B., Picard, R. G., and Wyckoff, R. W. G.: Studies of the Action of Sodium Fluoride on Human Enamel by Electron Microscopy and Electron Diffraction, *Pub. Health Rep.* **65**: 43, 1950.
6. Frank, R.: Etudes sur l'infrastructure microscopique de l'email et sur la surface libre coronaire de la dent. *Schweiz. Monatschr. f. Zahnheilk.* **60**: 1109, 1950.
7. Newman, S. B., Borysko, E., and Swerdlow, M.: Ultra-microtomy by a New Method. *J. Res. Nat. Bur. Standards* **43**: 183, 1949.

8. Sognnaes, R. F.: The Organic Elements of the Enamel. II. The Organic Framework of the Internal Part of the Enamel, with Special Regard to the Organic Basis for the So-Called Tufts and Schreger's Bands, *J. D. Res.* **28**: 549, 1949.
9. Latta, H., and Hartman, J. F.: Use of a Glass Edge in Thin Sectioning for Electron Microscopy, *Proc. Soc. Exper. Biol. & Med.* **74**: 436, 1950.
10. Wyckoff, R. W. G.: Electron Microscopy. New York, 1949, Interscience Publishers, p. 64.
11. Hopewell-Smith, A.: Evidence Against the Theory of the Metabolic Properties of Human Enamel, *New Zealand D. J.* **27**: 54, 1931.
12. Keil, A.: Beitrage zur Kenntnis der Doppelbrechung des menschlichen Zahnsubstanzes, *Zeitschr. f. Zellforsch.* **25**: 204, 1936.
13. Thewlis, J.: The Submicroscopic Structure of Dental Enamel, *D. Record* **60**: 49, 1940.
14. Sognnaes, R. F.: The Organic Elements of the Enamel. I. A Study of the Principal Factors Involved in the Histological Preservation of the Organic Elements of the Enamel and Other Highly Calcified Structures, *J. D. Res.* **27**: 609, 1948.
15. Frisbie, H. E., Nuckolls, J., and Saunders, J. B. deC. M.: Distribution of the Organic Matrix of the Enamel in the Human Tooth and Its Relation to the Histopathology of Caries, *J. Am. Coll. Dent.* **11**: 243, 1944.
16. Moller, H., and Trommel, G.: Roentgenographische Untersuchung uber den Aufbau der anorganischen Zahnsubstanz, *Naturwiss.* **21**: 346, 1933.
17. Bale, W. F., Hodge, H. C., and Warren, S. L.: Roentgen-ray Diffraction Studies of Enamel and Dentin, *Am. J. Roentgenol.* **32**: 369, 1934.
18. Thewlis, J.: The Examination of Dental Enamel by X-Rays and the Polarizing Microscope, *Proc. Phys. Soc. London* **51**: 992, 1939.

ELECTRON MICROSCOPY OF THE ENAMEL OF TEETH IN VARIOUS STAGES OF DEVELOPMENT

REIDAR F. SOGNNAES,* DAVID B. SCOTT,** MARIE J. USSING,*** AND RALPH W. G. WYCKOFF****

Harvard School of Dental Medicine, Boston, Mass.; and National Institutes of Health, Bethesda, Md.

IN HISTOLOGIC studies on the development of enamel a major distinction is usually made between the young matrix stage, in which the enamel remains relatively intact after demineralization, and the mature stage, in which the enamel has been characterized as acid-soluble, because it usually disappears completely during demineralization.[1] Technical advances have made it increasingly evident that the fully mature enamel actually retains an organic framework, the successful recovery of which requires various intricate methods of demineralization.[2] It is now apparent that this framework cannot be fully visualized under the optical microscope, for through electron microscopy it has recently been demonstrated that, in addition to the prism sheaths, there is a submicroscopic fibrillar network permeating the substance both within and between the prisms.[3]

A preceding report has described in detail the information gained by applying the thin sectioning and replica techniques to the electron microscopy of fully mature enamel.[3] The present paper is concerned with the electron microscopic appearance of enamel in various stages of development, as observed in thin sections. Special regard has been given to the changes occurring in the organic matrix during calcification.

MATERIAL AND METHODS

Specimens have consisted of molars and incisors dissected from the jaws of 1- to 12-day-old mice, and small pieces of enamel dissected from developing teeth in monkeys up to 3 years of age. They were embedded in plastic, cut an a Spenser rotary microtome with a thermal expansion adapter[4] with a glass knife.[5] Details of the procedures for embedding and sectioning dental tissues have been described in a previous paper.[3] The finished sections were shadowed lightly with palladium to enhance their stability under the electron beam.

Mouse teeth, because of their small size, are especially well suited to the requirements of the thin sectioning methods. Whole teeth can be embedded, and the areas of the resultant sections are small enough so that each one can be placed on a single specimen screen and examined in its entirety under the electron microscope. The intactness of these sections is demonstrated in Fig. 1. Larger teeth, such as those from monkeys and man, cannot be sectioned in this fashion, but must first be cut into small pieces of the proper size for subsequent embedding and microtomy.

Received for publication, Aug. 3, 1951.

*Harvard School of Dental Medicine.

**National Institute of Dental Research.

***National Institute of Dental Research, Present address: National Dental College of Denmark, Copenhagen.

****National Institute of Arthritis and Metabolic Diseases.

In the present study cross and longitudinal sections were cut through molar crowns, and cross-sections through incisors, from 1, 4, 7, and 12-day mice. Each embedding was cut at several different levels, thereby providing sections from different regions of the developing teeth. This was of special value in the case of the incisors, as quasi-serial cross-sections could be made along a tooth, from the developing zone to the incisal region.

The pieces of monkey enamel were dissected from various regions of developing permanent and deciduous tooth crowns, which had previously been removed from unborn, and 1 and 3-year-old animals. Most of the pieces were taken from incisal or occlusal, and cervical portions of canines and molars. Calcification was incomplete enough so that the enamel could be cut or shaved off with a sharp knife.

It was found that most molar crowns from 1 to 7-day-old mice, and the growing zone of incisors from mice of any age, could be sectioned without preliminary demineralization. The more fully calcified molars from older mice, and the portions of incisors external to the growing zone, had to be demineralized before they could be sectioned. Certain of the samples of monkey enamel could also be cut without demineralization; but, because the degree of calcification could not be estimated within as close limits as in the case of the mouse teeth, it was difficult to predict in advance whether or not sectioning would be successful. For purposes of comparison, specimens identical to those cut in undemineralized form were also demineralized prior to sectioning.

Mouse teeth were demineralized by two methods. Most of them were immersed for 12 to 72 hours in Zenker's acetic acid fluid, which was useful because fixation and demineralization were accomplished simultaneously. A few were first fixed for at least 24 hours in 4 per cent neutral formalin, and then demineralized by immersion for 8 to 24 hours in 5 per cent trichloracetic acid at low temperature, with and without elevated pressure.[2] The isolated monkey enamel was fixed and demineralized by the second method.

As a preliminary to electron microscopy sample sections were always first observed under the optical microscope. These sections were mounted on glass microscope slides, and were either left unstained, or were stained with hematoxylin and eosin, methylene blue, or Shorr's modified Masson trichrome stain.[6] The orientation of the sections in respect to the tooth from which they had been cut was thus made clear, and an impression was gained of the state of maturation of the enamel. A rough check on the presence or absence of crystalline material, and the degree of calcification, was made through use of polarized light and by electron diffraction. Diffraction was performed in an RCA type EMD apparatus. With the lenses of the instrument arranged so as to provide a shadow image of the specimen, it was possible to scan the section and select recognizable fields for diffraction.

Examinations under the electron microscope (RCA type EMU) were first made at low magnification (800×), which was obtained by removing the projector pole piece and insert and operating this lens at maximum current. Subsequent microscopy at higher magnification (3000-10,000×) was carried on with the lenses in standard operating arrangement. About 700 sections, made from 50 blocks, were examined, and more than 2000 micrographs taken.

RESULTS

For general orientation, Fig. 1 shows an unstained longitudinal section of a developing mouse molar crown, photographed at low magnification by transmitted light. The enlarged view of the lingual half of this molar illustrates the transitional stages in enamel maturation which can be studied in such preparations. The darker, less transparent, portions represent the youngest enamel. The increasing transparency toward the dentin surface, and toward the occlusal level, indicates progressively more advanced maturation in both directions.

Fig. 2 is an electron micrograph of the cervical portion of the enamel shown in Fig. 1, taken at low magnification. In the lower left corner a small triangle of dentin and well-defined dentino-enamel junction can be seen. The internal, older portion of the enamel, which approximates the dentin, is somewhat fibrillar, and it is structurally quite different from the relatively homogeneous, more recently formed, enamel shown to the right in the same illustration.

Fig. 1.—Unstained longitudinal section of Zenker-fixed, demineralized, developing molar from a 12-day-old mouse, photographed under the optical microscope. Insert—×50; enlarged lingual aspect—×175. (All other illustrations are electron micrographs.)

A homogeneous appearance of newly formed enamel, similar to that observed in the molar sections, can be seen in the left half of Fig. 3, which shows the outermost edge of a cross-section through the growing end of a mouse incisor. Where the matrix is in direct proximity to the enamel-forming ameloblasts, as shown in the right half of the picture, the distal ends of the cells contain large globules of amorphous material within their cytoplasm. Structurally these globules appear similar to the substance within the adjoining part of the enamel. Their significance is not yet known, but they may possibly represent the first step in matrix formation.

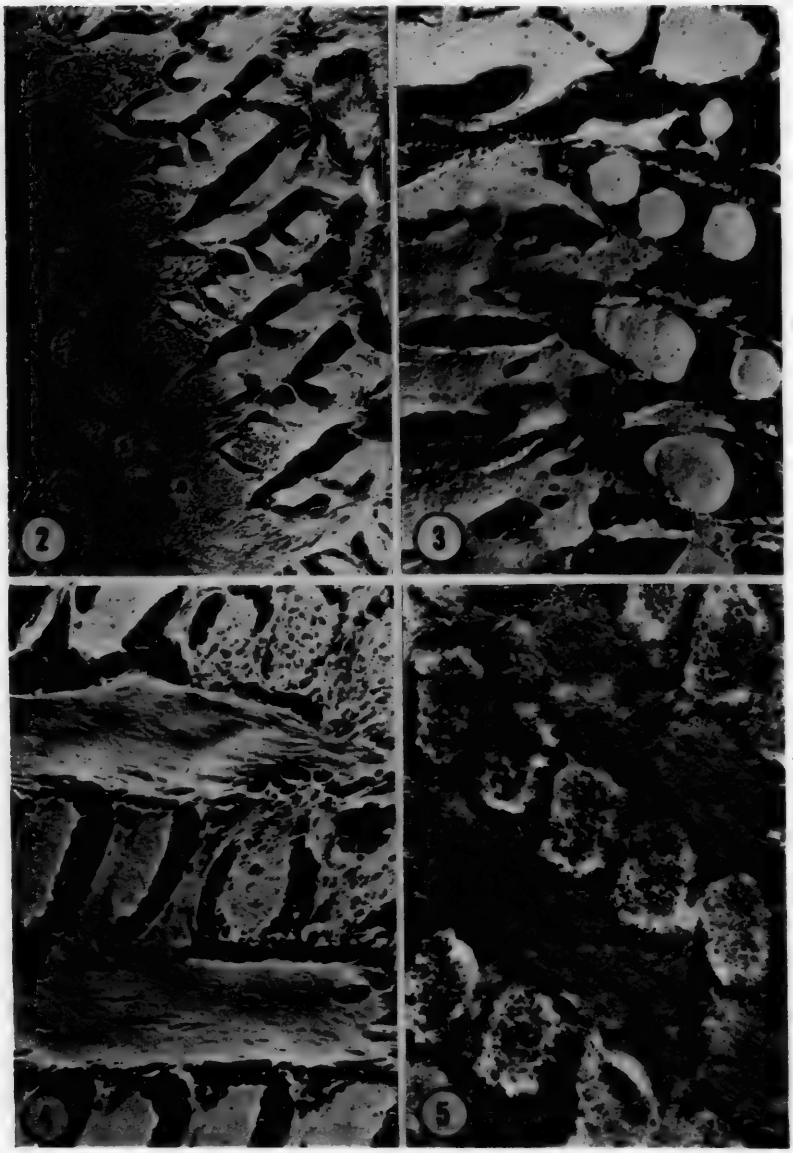

Fig. 2.—Cervical area of section seen in Fig. 1, showing, from right to left: young, homogeneous, and more mature, fibrillar enamel matrix. ×1,700.

Fig. 3.—Homogenous newly formed enamel matrix (left) and amorphous globules in cytoplasm of ameloblasts (right). ×7,200. This figure and Figs. 4, 6, 7, and 9 are all micrographs from cross-sections cut at different levels, from the apical growing zone to the fully mature incisal region, in the same tooth, a Zenker-fixed, demineralized incisor from a 12-day-old mouse.

Fig. 4.—Transitional zone between homogeneous and fibrillar matrix. ×7,200.

Fig. 5.—Similar developmental stage to that shown in Fig. 4, in formalin-fixed, undecalcified mouse enamel. ×5,800.

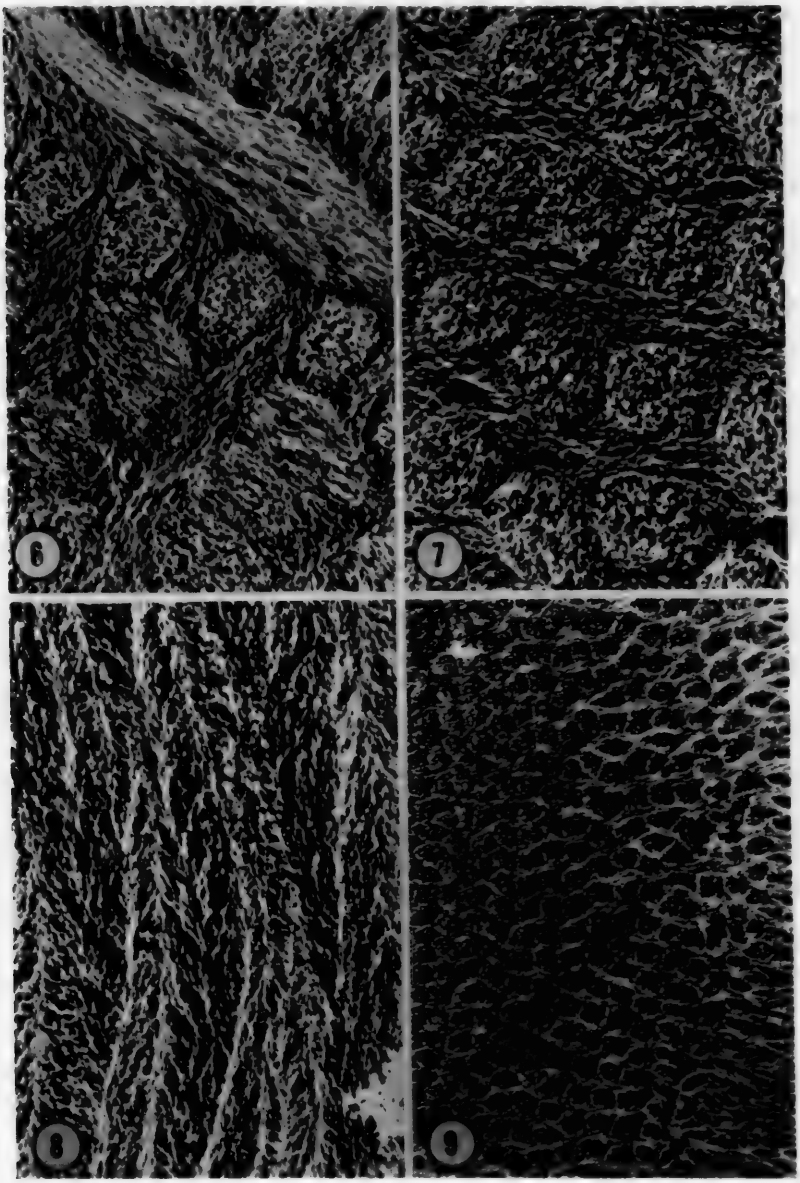

Fig. 6.—Closely packed fibrils in matrix of nearly mature enamel. ×7,200.

Fig. 7.—More widely spaced fibrils in mature enamel. ×7,200.

Fig. 8.—Fibrillar network in a longitudinal section of the mature enamel of a Zenker-fixed, demineralized molar, from a 12-day-old mouse. ×5,800.

Fig. 9.—Larger area of section seen in Fig. 7, photographed at low magnification, to show over-all fibrillar character of mature enamel matrix. ×1,700.

Between the homogeneous young enamel matrix and the fibrillar older matrix just mentioned is a transitional zone, in which the beginnings of fibril formation are evident. The appearance of this zone in sections of enamel fixed, and thereby demineralized, in Zenker's fluid is shown in Figs. 2 and 4. In sections of undemineralized enamel, fixed in neutral formalin, the region showed similar suggestions of beginnig fibril formation, as can be seen in Fig. 5. However, it was noted in these preparations that empty spaces, comparable to those in the Zenker-fixed enamel, were not evident. Whether or not these voids are due to a deleterious effect of acid fixation on the tissue is not defininitely known, but it is unlikely that at this stage in development of the enamel they are regions from which inorganic material has been dissolved away. It should be mentioned that such differences between Zenker-fixed and undemineralized enamel were observed only in the homogeneous and transitional regions.

In regions where the transition from homogeneous to fibrillar matrix has recently been completed, and traces of homogeneous substance are no longer visible, the fibrils are very closely packed together, as shown in Fig. 6. This fibril pattern seems to correspond roughly to the midpoint in enamel development. Its position, especially within molar enamel, depends upon the age of the specimen being examined. That it is in a region where appreciable calcification has occurred is clear from the fact that enamel had to be demineralized in order for it to be revealed satisfactorily.

The fibrils in fully-developed, completely calcified, enamel are no longer closely packed, but instead are widely spaced and apparently thinner. This is illustrated in Figs. 7 to 9. The resemblance of the fibrillar network permeating the entire substance of the enamel to that previously observed[3] in demineralized fully mature human enamel is particularly striking in Figs. 8 and 9.

While considerable basic information on the progressive changes which occur in the organic matrix of developing enamel has been obtained from examination of sections of mouse teeth, the dissimilarity between the prism configurations of rodent and human enamel in this type of preparation has precluded complete comparisons. In fact, differentiation between prismatic and interprismatic substance in mouse enamel was quite difficult, especially in younger specimens. The "herring-bone" appearance so characteristic of young rodent enamel, and the odd prism distribution noted in more mature enamel generally have been attributed to irregular arrangement of the prisms with respect to each other, in a fashion quite different from the parallel arrangement typical of human enamel.[7] It therefore seemed important, as a next step, to extend the studies to include developing monkey enamel, in which the prism relationships are similar to those in human enamel.

Observation of monkey enamel in various stages of development has indicated that the same progression of changes from homogeneous to fibrillar structure occurs as in the mouse enamel. The most interesting sections were obtained from enamel in which calcification had progressed to a point where sectioning in the undemineralized state was just barely possible. Figs. 10 to 13 provide a comparison of undemineralized and demineralized enamel of this type. Considerable damage is apparent in sections of the undemineralized enamel, as indicated by the discontinuity of structures in Figs. 10 and 11. Never-

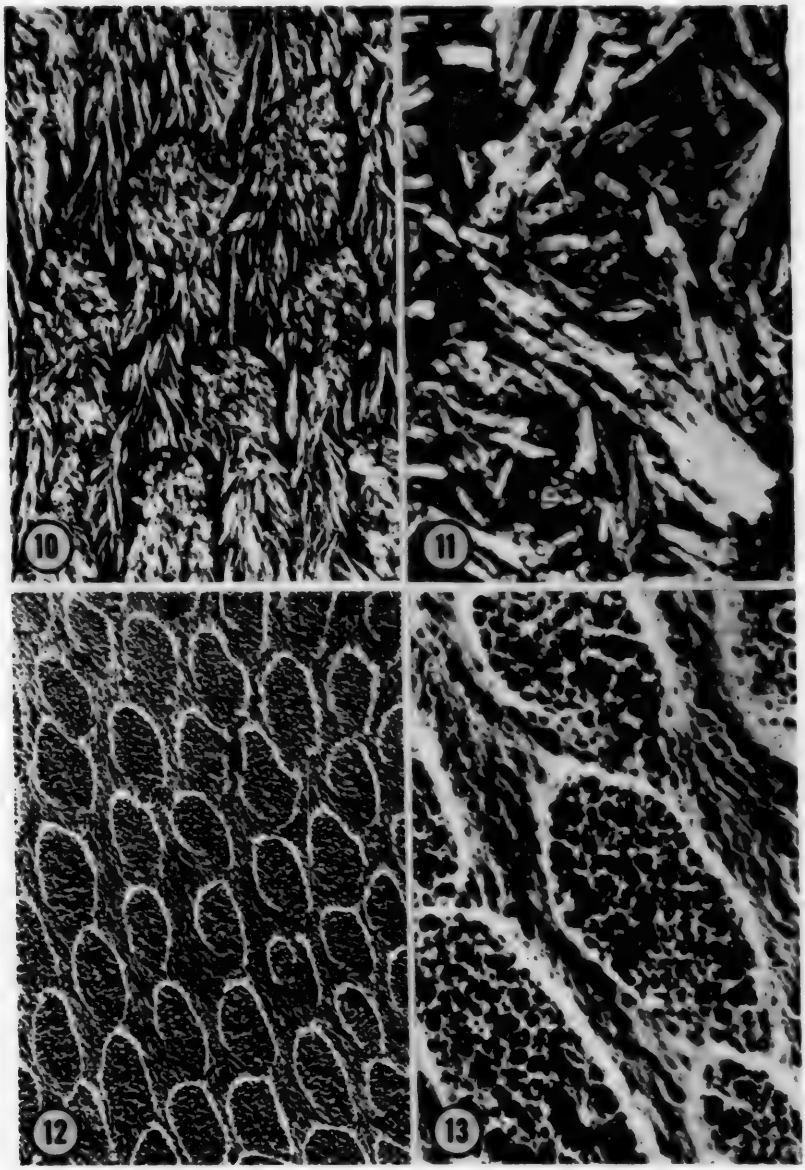

Fig. 10.—Crystal-like particles in a cross-section of undemineralized enamel from the cervical region of a developing third molar from a 3-year-old Rhesus monkey. ×1,700.

Fig. 11.—Crystal-like particles in an area at the periphery of the section shown in Fig. 10. ×14,800.

Fig. 12.—Cross-section of demineralized enamel from another third molar from the same monkey as above, showing prism sheaths and a submicroscopic fibrillar network between and within the prisms. ×1,700.

Fig. 13.—Apparently more advanced maturation in the prism cores than in the interprismatic substance, shown in a cross-section of demineralized incisal enamel of a developing canine from a monkey fetus. ×7,200.

theless, the general contours of the prisms could be distinguished, and minute crystal-like objects were noted both within and between the prisms. The appearance of such enamel at low magnification is shown in Fig. 10. It can be seen that there is considerable variation in the sizes and shapes of the crystal-like objects. In some regions, usually around the periphery, the particles were widely separated, obviously as a result of dispersion during preparation of the sections. Here they could be studied at higher magnification (Fig. 11), and had the external appearance of single crystals, parallel aggregates, and possibly cleavage fragments. More work is needed for their positive identification, but it was established that the enamel from which the preparations were made contained crystalline, acid-soluble material. When examined under polarized light it was strongly birefringent, whereas the same enamel when demineralized did not show this property. The presence of crystalline material was further demonstrated by electron diffraction. The exceptionally well-defined apatite patterns from sections of the undemineralized enamel were similar to those ordinarily given by fully mature human enamel. No evidence of crystallinity was forthcoming when sections of demineralized enamel were subjected to electron diffraction.

The appearance of demineralized monkey enamel in the same stage of development as above is shown in Figs. 12 and 13. At a relatively low magnification, in Fig. 12, a very delicate fibrillar network can be noted between and within the prisms, which are outlined by dense bands that undoubtedly are the prism sheaths. At higher magnification, in Fig. 13, a better distinction can be made between the prism core and the interprismatic substance. The organic matter within the core of the prisms is organized in a spongelike network resembling that found in fully mature enamel, while the interprismatic substance has an appearance nearer that of the matrix at an early stage in development. This could be taken to indicate that calcification within the prisms may have been slightly more advanced than between them. Such differences between the intraprismatic and interprismatic substance are not evident in the organic framework of demineralized fully mature human enamel.[3] Instead, a uniformly fibrillar network is present both between and within the prisms.

DISCUSSION

It is reasonable to conclude from the present evidence that the submicroscopic network which underlies mature enamel is not fully developed as such when the matrix is first laid down, but forms as the inorganic crystals are deposited. It is not known if the alterations in the organic matrix are directly caused by the ingress and expanding growth of the crystalline material, or are expressions of chemical changes that provide spaces for the forming crystals. Several chemical studies, however, have shown that the volume of the organic matter is gradually reduced as calcification progresses. By determining the content of water, inorganic and organic matter in enamel on a volumetric basis, Deakins[8, 9] was able to demonstrate that the influx of ash during calcification is compensated for chiefly by loss of water. A loss of organic matter itself was evident only during the earliest phase of calcification. It was further shown[9] that the Ca, P, and CO_2 contents increase linearly and in constant ratios to

each other as calcification progresses. Buck[10] has recently observed that similar chemical changes occur in pathologic calcification (atherosclerotic aortas). In specimens ranging from normal to a high degree of calcification he found that there was a logarithmic rate of increase in mineral concentration, a constant ratio of calcium to phosphorus, and, finally, that the influx of minerals was accompanied by loss of water. The proportion of fat-free organic residue remained unchanged. Applied to the findings in the present study, the above chemical data suggest that the homogeneous enamel matrix changes to its final fibrillar form through a process of dehydration and condensation, rather than through loss of the organic substance.

To date it has not been possible, even through replica studies, to observe the inorganic crystals in fully mature enamel, and therefore, measurements of crystal size still cannot be made in direct fashion. Many of the particles, like those of Fig. 11, observed in the sections of undemineralized developing monkey enamel, however, have had dimensions much smaller than those calculated from x-ray diffraction patterns for the individual crystals in mature enamel. Unless it can be shown that they are not bits of larger crystals fragmented during specimen preparation, their significance is not clear. Similarly minute crystal-like particles have also been observed under the electron microscope by Boyle, Hillier, and Davidson,[11] in suspensions of developing enamel which had been dispersed in collodion by digital pressure. They thought that some of their particles were fragments of comminuted larger crystals.

SUMMARY

Thin sections for electron microscopy have been made from demineralized and undemineralized mouse and monkey enamel in various stages of development. Electron micrographs are presented which demonstrate a change from homogeneous to fibrillar structure which takes place in the organic matrix during maturation. Minute crystal-like particles have been noted in sections of undemineralized, partly calcified, monkey enamel.

REFERENCES

1. Diamond, M., and Weinmann, J. P.: The Enamel of Human Teeth, New York, 1940, Columbia Univ. Press.
2. Sognnaes, R. F.: The Organic Elements of the Enamel. I, II, III, IV. *J. D. Res.* **27**: 609, 1948; **28**: 549 and 558, 1949; **29**: 260, 1950.
3. Scott, D. B., Ussing, M. J., Sognnaes, R. F., and Wyckoff, R. W. G.: Electron Microscopy of Mature Human Enamel, *J. D. Res.* **31**: 74, 1952.
4. Newman, S. B., Borysko, E., and Swerdlow, M.: Ultra-Microtomy by a New Method, *J. Res. Nat. Bur. Standards* **43**: 183, 1949.
5. Latta, H., and Hartman, J. F.: Use of a Glass Edge in Thin Sectioning for Electron Microscopy, *Proc. Soc. Exper. Biol. & Med.* **74**: 436, 1950.
6. Shorr, E.: Evaluation of Clinical Application of Vaginal Smear Method, *J. Mt. Sinai Hosp.* **12**: 667, 1945.
7. Orban, B., Sicher, H., and Weinmann, J. P.: Amelogenesis, *J. Am. Coll. Dent.* **10**: 13, 1943.
8. Deakins, M.: Changes in the Ash, Water, and Organic Content of Pig Enamel During Calcification, *J. D. Res.* **21**: 429, 1942.
9. Deakins, M., and Burt, R. L.: The Deposition of Calcium, Phosphorus, and Carbon Dioxide in Calcifying Dental Enamel, *J. Biol. Chem.* **156**: 77, 1944.
10. Buck, R. C.: Minerals of Normal and Atherosclerotic Aortas, *Arch. Path.* **51**: 319, 1951.
11. Boyle, P. E., Hillier, J., and Davidson, N. R.: Preliminary Observations of the Enamel of Human and Guinea Pig Teeth Using the Electron Microscope, *J. D. Res.* **25**: 156, 1946.

THE EFFECT OF TOPICAL APPLICATIONS OF SODIUM FLUORIDE ON THE ORAL FLORA OF YOUNG ADULTS

NORMAN H. RICKLES, D.D.S., M.S., AND HERMANN BECKS, M.D., D.D.S.

From the Division of Dental Medicine, College of Dentistry and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.

THE reduction of dental caries incidence by the topical application of sodium fluoride to the teeth of children has been demonstrated by several investigators.[2, 5, 6, 11-14] This effect on dental caries may also be true for young adults as reported by Klinkenberg and Bibby,[10] and Rickles and Becks.[18] It would be of more than academic interest if the mechanism of this action could be clarified.

Several investigators have shown that enamel is less soluble in acid after the application of fluorides.[3, 16, 17] However, this decreased solubility may only partly explain the caries-reducing effect of sodium fluoride. There is some evidence to indicate that the fluoride ion is an effective enzyme inhibitor. Unfortunately, the mechanism of its release from the bound molecular or crystalline structure in the enamel is at present not clear. Assuming that bacterial acid production is essential in the pathogenesis of dental caries, it is possible that the fluoride ion's constant activity could interfere with the phosphatases of the bacterial enzyme system, thus preventing the breakdown of refined carbohydrates to various acids. Furthermore, it is also conceivable that the fluoride ion could prevent the denaturation of the lamellar proteins by paralyzing the bacterial enzyme systems necessary for this process.

Bibby[1] has shown that fluoride ions in sufficient strength in culture media inhibit the growth of lactobacilli, and in much weaker concentration greatly lessen their acidogenic properties. Clapper[4] found that lactobacilli maintained in a medium containing 100 p. p. m. of NaF developed reduced ability to produce acid from glucose even when returned to a fluoride-free medium. Kitchin, Robinson, Permar and Imhoff[9] demonstrated no significant decrease in lactobacillus colony counts after topical applications of a 2 per cent solution of NaF.

The purpose of this study is to determine the effect of topically applied sodium fluoride on the acidogenic properties of the total oral flora of young adults and on the *Lactobacillus acidophilus* index.

MATERIAL

A total of 143 subjects were chosen at random from the student body of the University of California. They ranged in age from 18 to 34 years, with an average age of 22 years. A 2 per cent solution of sodium fluoride C.P. in distilled water was applied to the teeth of 97 subjects once a week for four weeks,* while 46 control individuals were treated with distilled water in the same manner.

Aided by grants from the Regents of the University of California, the California State Dental Association, the American Foundation for Dental Science and the Grant from the office of the Surgeon General, War Department (#W-49-007-MD-470).

Received for publication, Aug. 25, 1951.

*According to the technique recommended by the United States Public Health Service (15).

Two days before beginning the applications and again one week after their completion, the following procedures were performed for all 143 subjects: 1. An early morning paraffin-stimulated saliva sample was collected. 2. The pH of the individual saliva sample when mixed with a sucrose-indicator solution* was determined hourly by comparison with standard color tubes.†

PROCEDURE

The saliva (0.5 c.c.) to be tested was added to 0.5 c.c. of the above solution in a precipitation tube, bringing the concentration of sucrose to approximately 3.9 per cent. This made sufficient sucrose available for degradation to acid by oral bacterial enzyme systems, yet did not produce a great enough osmotic pressure to inhibit the metabolism of these microorganisms. This saliva-sucrose indicator mixture (hereafter referred to as the saliva-sucrose mixture) was then incubated at 37° C. The change in pH was determined by comparison with the standard buffer-indicator solutions and recorded each hour for seven hours. The total amount of acid produced at the end of 7 hours was found by titrating the washed contents of the sample tube with 0.02 N sodium hydroxide using a Beckman pH meter set at pH 6.6 (The original pH of each test sample). The L.a. index was determined from the same saliva sample.[8]

RESULTS

1. *pH Changes in Saliva-Sucrose Mixture.—*

Experimental group.—Individual pH changes after NaF applications were recorded at 0.2 pH intervals.‡ A frequency distribution is seen in Table I.

At the end of a one hour incubation period, 39 of the 97 samples showed no change whatsoever in pH (Xe = 0). A pH higher by 0.2 interval occurred in 8 cases, while 6 demonstrated an equivalent drop. Five samples disclosed an increase in pH of 0.8 and three a corresponding decrease (Xe = +8 and –8, respectively). After 5 hours incubation, 30 samples still did not show any change; however, the extremes after this time were much greater than at 1 hour, with 2 samples demonstrating an increase of 1.6 pH units and 1 showing a decrease of 2.0 units. Generally, it was noticed that an increase in the extremes of pH differences occurred with increasing time of incubation. The values for ΣT_e, $\Sigma T_e X_e$, $\Sigma T_e X_e^2$, ΣX_e, M_e, ΣX_{te}, and M_{te} (Table I) were computed from the foregoing data and used for statistical evaluation.

Control group:

The frequency distribution of pH changes is shown in Table II.

*The sucrose-indicator solution was made up by adding to 1000 c.c. of distilled water 80 Gm. of sucrose, 10 c.c. Brom-cresol-purple (0.4%) and 10 c.c. Brom-cresol-green (0.4%). The pH was adjusted to 6.6 with 4 cc. of $\frac{N}{20}$ NaOH.

†Standard phosphate and phthalate-HCl buffer solutions were made up at 0.4 pH intervals, ranging from pH 6.6 to pH 3.3 One c.c. of these buffers was placed into small precipitation tubes to which 0.1 c.c. of a 0.04% solution of Brom-cresol-purple and 0.1 c.c. of a 0.04% solution of Brom-cresol-green were added. Sharp color changes in the standard solutions at 0.4 pH intervals resulted

‡The pH's of the saliva-sucrose samples after the applications were subtracted from the values before treatment to obtain the difference or change in pH.

TABLE I. EXPERIMENTAL GROUP: FREQUENCY DISTRIBUTION OF CHANGES IN pH FOLLOWING ONE TO SEVEN HOURS OF INCUBATION

CHANGES IN pH (X_e)	HOURS 1	2	3	4	5	6	7	ΣT_e	$\Sigma T_e X_e$	$\Sigma T_e X_e^2$
2.0										
1.8										
1.6					2	1	2	5	8.0	12.8
1.4						1		1	1.4	1.96
1.2		1		3	5	5	2	16	19.2	23.04
1.0					1			1	1.0	1.0
0.8	5	5	8	11	9	7	6	51	40.8	32.64
0.6	3	1	3	2	2			11	6.6	3.96
0.4	21	26	27	18	9	17	18	136	54.4	21.76
0.2	8	4	5	3				20	4.0	0.8
0.0	39	27	20	25	30	20	23	184	0	0
−0.2	6	5	4	1				16	−3.2	0.64
−0.4	11	11	9	10	16	28	25	110	−44.0	17.6
−0.6	1	3	3	2			1	10	−6.0	3.6
−0.8	3	9	10	14	11	12	12	71	−56.8	45.44
−1.0			1	1		1		3	−3.0	3.0
−1.2		5	3	3	10	3	4	28	−33.6	40.32
−1.4										
−1.6			4	4	1	2	4	15	−24.0	38.4
−1.8										
−2.0					1			1	−2.0	4.0
ΣX_e	7.2	−3.4	−5.2	−6.2	−8.6	−7.2	−13.8	−37.2*		250.96
M_e	0.0742	−0.035	−0.0536	−0.0639	−0.0887	−0.0742	−0.142	−0.0548†		

ΣT_e = The sum of the frequencies of the X_e's at the different hours
$\Sigma T_e X_e = (\Sigma T_e)(X_e)$
$\Sigma T_e X_e^2 = (\Sigma T_e X_e)(X_e)$
*ΣX_{te}, †M_{te}, M_e ΣX_e, (Identified in Table III)

TABLE II. CONTROL GROUP: FREQUENCY DISTRIBUTION OF CHANGES IN pH FOLLOWING ONE TO SEVEN HOURS OF INCUBATION

CHANGES IN pH (X_c)	HOURS 1	2	3	4	5	6	7	ΣT_c	$\Sigma T_c X_c$	$\Sigma T_c X_c^2$
2.0										
1.8										
1.6										
1.4										
1.2					1	1		2	2.4	2.80
1.0						1		1	1.0	1.00
0.8	3	1	3	6	5	2	3	23	18.4	14.70
0.6		4	1	2	1	1		9	5.4	3.24
0.4	8	6	10	6	10	12	10	62	24.8	9.91
0.2	1	2	2	2				7	1.4	0.28
0.0	22	14	11	7	10	7	14	85	0.0	0.00
−0.2	2	3	1	2				8	−1.6	0.32
−0.4	9	12	6	10	8	13	11	69	−27.6	11.10
−0.6			1	1	1			3	−1.8	1.08
−0.8		2	8	6	3	1	3	23	−18.4	14.70
−1.0					2			2	−2.0	2.00
−1.2	1	1	1	3	3	6	3	18	−21.6	26.00
−1.4					1			1	−1.4	1.96
−1.6		1	2	1	1	2	2	9	−14.4	23.00
−1.8										
−2.0										
ΣX_c	0.6	−3.8	−6.6	−6.2	−5.0	−7.2	−7.2	−35.4*		112.09
M_c	0.013	−0.0826	−0.143	−0.1347	−0.1086	−0.1565	−0.1565	−0.1098†		

ΣT_c = The sum of the frequencies of the X_c's at the different hours
$\Sigma T_c X_c = (\Sigma T_c)(X_c)$
$\Sigma T_c X_c^2 = (\Sigma T_c X_c)(X_c)$
*ΣX_{tc}, †M_{tc}, ΣX_c, M_c, –Identified in Table III.

These data are similar to those of the former group. Here the extremes occurred at the completion of the 6 hour incubation period when 1 mixture demonstrated an increase of 1.2 pH units and 2 samples showed a decrease of 1.6 units. The results obtained for ΣT_e, $\Sigma T_e X_e$, $\Sigma T_e X_e^2$, ΣX_e, M_e, ΣX_{te}, and M_{te} were again computed for statistical evaluation.

The sum of all the individual changes in pH, mean pH changes for each hour, and the total means for the 7 hours are summarized in Table III for both groups.

TABLE III. HOURLY CHANGES IN pH (SUMS & MEANS) AFTER TREATMENT

HOURS	ΣX_e	ΣX_c	M_e	M_c
1	7.2	0.6	0.0742	0.013
2	−3.4	−3.8	−0.035	−0.0826
3	−5.2	−6.6	−0.0536	−0.143
4	−6.2	−6.2	−0.0639	−0.1347
5	−8.6	−5.0	−0.0887	−0.1086
6	−7.2	−7.2	−0.0742	−0.1565
7	−13.8	−7.2	−0.142	−0.1565
Totals	ΣX_{te}	ΣX_{tc}	M_{te}	M_{tc}
	−37.2	−35.4	−0.0548	−0.1098

ΣX_e = The sum of the changes in pH of the saliva-sucrose mixture of the experimental group after the application of the 2 per cent NaF solution.

ΣX_c = The sum of the changes in pH of the saliva-sucrose mixture of the control group after the application of water.

$M_e = \frac{\Sigma X_e}{N_e}$ = The mean changes in pH of the saliva-sucrose mixture of the experimental group after the application of the 2% NaF solution.

$M_c = \frac{\Sigma X_c}{N_c}$ = The mean change in pH of the saliva-sucrose mixture of the control group after the application of the water.

$\Sigma X_{te} = \Sigma(\Sigma X_e\text{'s})$ = The sum of all the ΣX_e's for the 7 hours.

$\Sigma X_{tc} = \Sigma(\Sigma X_c\text{'s})$ = The sum of all the ΣX_c's for the 7 hours.

M_{te} = Total mean of the experimental group for the 7 hours $= \frac{\Sigma X_{te}}{N_{te}}$

M_{tc} = Total mean of the control group for the 7 hours $= \frac{\Sigma X_{tc}}{N_{tc}}$

N_e = Total number of individuals in the experimental group = 97

N_c = Total number of individuals in the control group = 46

N_{te} = Total number of observations in the experimental group = (N_e) (hours) = (97) (7) = 679

N_{tc} = Total number of observations in the control group = (N_c) (hours) = (46) (7) = 322

It is seen that pH values (ΣX_e, ΣX_c, M_e, and M_c) of the saliva-sucrose mixtures of both groups tended to decrease with increasing time of incubation. However, the mean pH changes after treatment were not as low for the experimental group as for the control group. This variation between the two groups is demonstrated graphically in Fig. 1.

During the first hour of incubation a mean increase in the pH changes occurred in both groups, followed by steadily decreasing values for the remaining 6 hours. The slopes of the two curves were roughly parallel and at a fairly constant distance from each other; however, the values for the NaF group were consistently less acid than those for the control group.

2. *L.a. Index and Total Titratable Acid.*—

The individual values for L.a. indices and total titratable acid (in c.c.'s of 0.02 N NaOH) are shown, for each subject before and after applications of 2 per cent NaF or water, in Tables IV and V.

TABLE IV

L.a. INDICES AND TOTAL TITRATABLE ACID DETERMINED BEFORE AND AFTER APPLICATION OF 2 PER CENT NaF

NO.	L.a. INDEX		TOTAL ACID		NO.	L.a. INDEX		TOTAL ACID	
	BEFORE APPLICATIONS	AFTER APPLICATIONS	BEFORE APPLICATIONS	AFTER APPLICATIONS		BEFORE APPLICATIONS	AFTER APPLICATIONS	BEFORE APPLICATIONS	AFTER APPLICATIONS
1	11,000	2,500	0.5	0.6	50	0	0		
2	79,000	0	0.7	0.6	51	117,000	12,000		
3	405,000	43,000	1.3	0.3	52			0.5	0.5
4	6,000	11,200	0.7	0.5	53			0.5	0.6
5	50,000	2,000	0.4	0.6	54	0	312,000	0.6	0.5
6	5,200	15,000	0.4	1.0	55	0	1,500	0.6	0.3
7	10,000	6,000	0.4	0.8	56	202,000	83,000		
8	300,000	7,500	1.1	0.5	57	67,000	25,000		
9	12,000	0	0.7	0.8	58	34,000	0	0.5	0.5
10			0.9	0.6	59	40,000	20,000	1.3	0.9
11	0	0	0.8	0.4	60	5,000	3,000	1.2	0.5
12	101,000	11,000	0.9	0.6	61	62,000	100	0.5	1.0
13	27,000	131,000	0.5	0.5	62	600,000	187,000	0.8	1.1
14	190,000	120,000	0.4	0.4	63	40,000	32,500		
15	175,000	45,000	0.8	0.3	64	450,000	200,000		
16	37,000	600	0.8	0.3	65			0.4	0.7
17	0	0	0.5	0.9	66	0	0		
18	225,000	71,000	1.1	1.4	67	37,000	15,000		
19	8,000	500	0.4	0.6	68	3,000	30,000	0.8	0.7
20	340,000	0	0.5	0.5	69	14,000	150,000		
21	0	0	1.0	0.8	70	0	0		
22	8,000	13,000			71	200	0	0.6	0.7
23	100	200	0.0	0.8	72	35,000	0	0.4	0.7
24	13,000	0	0.3	0.8	73	390,000	31,000	1.2	0.7
25	2,700	22,500			74	12,000	45,000	1.1	0.6
26	20,000	0	0.5	0.5	75	100	0		
27	2,340,000	1,120,000			76	3,000	4,000		
28	2,200	1,000	0.5	0.4	77	270,000	0	0.4	0.6
29	3,000	6,700	0.6	0.4	78	62,000	20,000	0.4	0.7
30	16,000	52,000			79	10,200	20,000		
31	36,000	12,000			80	182,000	37,500	0.2	0.4
32	0	0			81	0	40,000	0.4	0.5
33	130,000	70,000			82	16,000	75,000		
34	450,000	175,000			83	0	0		
35	1,135,000	25,000	0.9	0.9	84	0	225,000	0.5	0.4
36	150,000	260,000	0.5	0.4	85	797,000	5,000	0.0	0.8
37	5,000	4,000	0.2	0.6	86	84,000	30,000	0.5	1.1
38	45,000	300			87	7,000	3,000	0.2	1.0
39	42,500	0	0.1	0.5	88	147,000	0	0.3	0.4
40	200	0			89	0	0	0.5	0.6
41	0	0			90	0	0	0.9	1.3
42	360,000	182,000			91	0	0	0.8	0.3
43	75,000	185,000			92	200	0		
44	60,000	20,000			93	385,000	327,000	0.8	0.8
45	105,000	7,000			94	50,000	0	1.3	0.5
46	0	0			95	40,000	0	0.0	0.5
47	227,000	15,000			96	57,500	0	0.5	1.0
48	200	1,000	0.4	0.6	97	122,000	3,270,000		
49	0	300							

No consistent pattern could be found. Of the 93 of the original 97 experimental individuals studied for L.a. indices, 59.2 per cent showed a decrease as compared with 54.4 per cent of the 46 in the control group, while 14 per cent and 15.2 per cent, respectively, showed the same index as before the applications. Increased counts occurred in 26.8 per cent of the experimental group as against

30.4 per cent in the controls. Some individuals showed a drop in their indices from high values (over 1,000) to zero (12.9 per cent and 17.4 per cent). These percentages approximated each other very closely and their differences were statistically insignificant.[7]

Furthermore, even though 37.7 per cent and 48.9 per cent, respectively, demonstrated decreases of total titratable acid after treatment, the difference between the two groups was not statistically significant.[7] In the NaF group, 11.5 per cent produced the same amount of acid as before treatment, against 4.5 per cent in the H_2O group. Increased acid was produced by 50.8 per cent of the experimental and 46.6 per cent of the control group.

The mean changes in the L.a. indices and total titratable acid for both groups are shown in Table VI; no significant differences were found.[7]

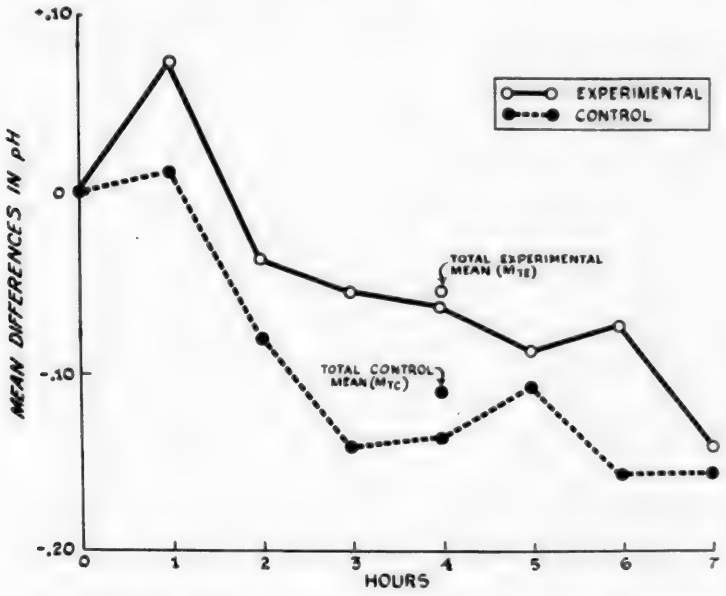

Fig. 1.—Mean changes in pH of salivary-sucrose samples after treatment, at hourly intervals.

DISCUSSION

At first glance the data seemed to demonstrate the fluoride ion's anti-enzymatic activity in that less acid is produced at each hour and in toto (M_{te} and M_{tc}) in the experimental than in the control group. However, on statistical evaluation of the mean differences at each hour and in toto between both groups, the resulting critical ratios were far below the values required for significance. Therefore, the trend illustrated in Fig. 1 was not significant, being possibly due to chance. The only reasonable inference is, that under the conditions of this investigation, the fluoride ion did not reduce the acidogenic properties of the oral flora.

TABLE V

L.a. INDICES AND TOTAL TITRATABLE ACID DETERMINED BEFORE AND AFTER APPLICATION OF WATER

NO.	L.a. INDEX		TOTAL ACID		NO.	L.a. INDEX		TOTAL ACID	
	BEFORE APPLICATIONS	AFTER APPLICATIONS	BEFORE APPLICATIONS	AFTER APPLICATIONS		BEFORE APPLICATIONS	AFTER APPLICATIONS	BEFORE APPLICATIONS	AFTER APPLICATIONS
1	140,000	27,000	1.0	0.8	24	35,000	225,000	0.1	1.1
2	32,500	0	0.5	0.4	25	56,000	7,000	0.3	0.4
3	275,000	450,000	0.6	1.1	26	87,500	5,000	0.8	1.0
4	37,000	0	1.3	0.9	27	1,000	0	1.0	0.8
5	0	0	0.5	0.7	28	20,000	7,000	1.0	0.6
6	0	0	0.3	0.4	29	52,500	50,000	1.2	0.4
7	237,000	0	0.1	0.2	30	30,700	170,000	0.3	0.4
8	475,000	600,000	0.1	1.1	31	190,000	40,000	0.4	0.5
9	0	0	0.4	0.6	32	50,000	90,000	0.8	0.6
10	425,000	468,000	0.7	0.6	33	77,000	225,000	0.3	0.4
11	52,000	7,000	1.4	0.9	34	50,000	5,000	0.9	0.3
12	37,200	4,000	0.8	0.4	35	175,000	53,000	0.7	0.5
13	375,000	300	0.3	0.2	36	18,700	30,000	1.0	0.6
14	81,000	0	0.3	0.6	37	5,000	5,000	1.0	1.2
15	127,000	10,000	0.5	0.4	38	37,500	0		
16	342,000	450,000	0.1	0.1	39	23,000	125,000	1.0	1.1
17	12,500	0	0.2	0.3	40	0	600	1.2	0.8
18	0	0	0.4	0.5	41	3,000	7,000	1.3	0.7
19	100	0	0.4	0.2	42	0	0	0.5	0.7
20	65,000	0	1.2	0.6	43	45,000	135,000	0.6	0.5
21	525,000	175,000	0.3	1.7	44	422,000	3,000	0.5	0.5
22	415,000	187,000	1.2	0.5	45	15,000	225,000	0.2	0.7
23	42,500	0	0.3	0.5	46	0	0	0.6	0.5

These findings were augmented by the observation that no significant difference existed between the mean changes in total titratable acid in both groups after the applications. Furthermore, the difference between the mean decreases in L.a. indices in the two groups were not statistically significant either.

Although these results do not seem to support the "enzyme-inhibitor" rationale of topically applied NaF, several factors may be acting to mask such an effect:

1. The microorganisms tested were those present generally throughout the mouth and therefore were only in intermittent contact with the fluoride ion. Although an inhibitory action of NaF under these in vitro conditions, which were optimal for acid production, could not be demonstrated, it is conceivable that in vivo, i.e., in the oral cavity, such an effect may occur.

TABLE VI

MEAN CHANGES IN L.a. INDICES AND TOTAL TITRATABLE ACID AFTER TREATMENT

GROUPS	L.a. INDICES			TOTAL TITRATABLE ACID (IN C.C.'S OF 0.02 N NaOH)		
	Σ*	N†	M‡	Σ*	N†	M‡
Sodium fluoride	−3,704,900	93	−39,850	+2.3	62	+0.0371
Water	−1,303,600	46	−28,400	−0.6	45	−0.0133

*Sum of all the changes (values after applications subtracted from those before). A minus sign (−) indicates a decrease in the total values after application of the NaF or water.

A plus sign (+) indicates an increase in the total values after application of the NaF or water.

†Number of individuals studied.

‡Mean change—plus and minus signs interpreted similarly to those for Σ.

2. Since most of the fluoride ions were presumably still on the tooth surfaces and only relatively few in the saliva-sucrose mixtures, it is doubtful that they could exert a sustained antienzymatic activity in vitro. Furthermore, it is obvious that the relatively short exposure of the oral flora to the fluoride ions on the teeth had not altered their ability to produce acid in vitro.

CONCLUSIONS

1. The application of a 2 per cent aqueous solution of NaF to the teeth of young adults did not alter: (1) the acidogenic properties of the oral flora; (2) the *Lactobacillus acidophilus* index.

2. The findings do not seem to support the "enzyme-inhibitor" explanation for the dental caries-reducing effect of topically applied NaF.

REFERENCES

1. Bibby, B. G., and Van Kesteren, M.: The Effect of Fluorine on Mouth Bacteria, *J. D. Res.* **19**: 391, 1940.
2. Bibby, B. G.: Use of Fluorine in the Prevention of Dental Caries. II. *J. A. D. A.* **31**: 317, 1944.
3. Buoncore, M. G., and Bibby, B. G.: The Effects of Various Ions on Enamel Solubility, *J. D. Res.* **24**: 103, 1945.
4. Clapper, W.: The Effect of Sodium Fluoride on the Metabolism of Oral Lactobacilli, *Proc. Soc. Exper. Biol. & Med.* **65**: 333, 1947.
5. Galagan, D. J., and Knutson, J. W.: Effect of Topically Applied Fluorides on Dental Caries Experience. V. Report of Findings with 2, 4, and 6 Applications of Sodium Fluoride and of Lead Fluoride, *U. S. Public Health Reports* **62**: 1477, 1947.
6. Galagan, D. J., and Knutson, J. W.: Effect of Topically Applied Fluoride on Dental Caries Experience. VI. Experiments with Sodium Fluoride and Calcium Chloride—Widely Spaced Applications—Use of Different Solution Concentrations, *U. S. Public Health Reports* **63**: 1215, 1948.
7. Garrett, H. E.: Statistics in Psychology and Education, ed. 3, New York, London, Toronto, 1947, Longmans, Green, and Co., Ch. 7, pp. 197-203, 219.
8. Hadley, F. P.: Quantitative Method for Estimating Bacillus Acidophilus in the Saliva, *J. D. Res.* **13**: 415, 1933.
9. Kitchin, P. C., Robinson, H. B. G., Permar, D., and Imhoff, A.: Observations on Salivary Lactobacillus Counts for a Period Before and After Topical Applications of 2 Per Cent Sodium Fluoride, *J. D. Res.* **30**: 251, 1951.
10. Klinkenberg, E., and Bibby, B. G.: The Effect of Topical Applications of Fluorides on Dental Caries in Young Adults, *J. D. Res.* **29**: 4, 1950.
11. Knutson, J. W., and Armstrong, W. D.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. II. Report of Findings for Second Study Year, *U. S. Public Health Reports* **60**: 1085, 1945.
12. Knutson, J. W., and Armstrong, W. D.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. III. Report of Findings for Third Study Year, *U. S. Public Health Reports* **61**: 1683, 1946.
13. Knutson, J. W., Armstrong, W. D., and Feldman, F. M.: The Effect of Topically Applied Sodium Fluoride on Dental Caries Experience. IV. Report of Findings with 2, 4, and 6 Applications, *U. S. Public Health Reports* **62**: 425, 1947.
14. Knutson, J. W.: An Evaluation of the Effectiveness as a Caries Control Measure of the Topical Application of Solution of Fluorides, *J. D. Res.* **27**: 340, 1948.
15. Knutson, J. W.: Sodium Fluoride Solution: Technique for Application to the Teeth, *J. A. D. A.* **36**: 37, 1948.
16. Muhler, J. C., and Van Huysen, G.: Sodium Fluoride Reagents Concentration and Powdered Enamel Solubility, *J. D. Res.* **27**: 142, 1948.
17. Muhler, J. C., and Van Huysen, G.: Solubility of Enamel Protected by Sodium Fluoride and Other Compounds, *J. D. Res.* **26**: 119, 1947.
18. Rickles, N. H., and Becks, H.: The Effects of an Acid and a Neutral Solution of Sodium Fluoride on the Incidence of Dental Caries in Young Adults. (In press.)

RELATION OF pH TO THE EFFECTIVENESS OF SODIUM FLUORIDE AND STANNOUS FLUORIDE IN DECREASING ENAMEL SOLUBILITY

JOSEPH C. MUHLER,* D.D.S., AND HARRY G. DAY, Sc.D.

Department of Chemistry, Indiana University, Bloomington, Ind.

PHILLIPS and Muhler[1] reported that the effectiveness of sodium fluoride in reducing the solubility of both powdered enamel and whole tooth sections is increased as the hydrogen ion concentration is increased. This was confirmed by Palmer, Overstreet, and Sacks[2] on the basis of laboratory evidence, but clinical evidence[3] failed to confirm it. The latter was based upon the topical application of sodium fluoride of different pH values.

The reason for the difference between the laboratory and clinical findings is evident when chemical principles are considered. The findings of Phillips and Muhler[1] and of Palmer, Overstreet, and Sacks[2] were based upon gravimetric procedures and, in principle, they were similar. In the method of the former workers[1] powdered enamel or whole tooth sections were immersed in a sodium fluoride solution containing 900 ppm fluorine. In the different experiments the pH of these solutions ranged from 2.6 to 7.5. After this treatment the enamel or whole tooth sections were isolated by filtration and then stirred for 20 minutes in 0.2 N acetic acid. Thereupon the solid phase was separated by filtration once more, washed, dried, and weighed. Control samples were subjected to the same treatment except that sodium fluoride was omitted. In this method, as well as in the method of Palmer, Overstreet, and Sacks,[2] most of the calcium dissolved in the presence of sodium fluoride must have been precipitated as calcium fluoride, which is highly insoluble in the pH ranges employed. Moreover, the lower the pH the more calcium was dissolved and the more calcium fluoride must have been precipitated. Thus it is reasonable to suppose that the relatively low loss in weight observed in the samples treated with sodium fluoride was due in part to the presence of the insoluble calcium fluoride.

Therefore, another experimental method was employed to reexamine the relation of pH to the effectiveness of sodium fluoride in decreasing the solubility of enamel. Also, owing to the promise of stannous fluoride as a highly effective agent for the protection of the teeth from dental caries,[4, 5] this compound was compared with sodium fluoride.

EXPERIMENTAL

The method of choice was substantially the same as that of Muhler, Boyd and Van Huysen[6] for the evaluation of the effectiveness of various compounds in decreasing the solubility of dental enamel, dentin, and tricalcium phosphate in weak acid. In this method a 100 mg. portion of powdered enamel[1] was added to 5 ml. of a fluoride solution the pH of which had been adjusted to the desired value with hydrochloric acid and sodium hydroxide. After shaking the mixture for 20 minutes at 25° C. it was promptly centrifuged. The clear

Supported in part by a grant from the Procter and Gamble Company.
Received for publication, June 2, 1951.
*Postdoctorate fellow of the National Institutes of Health.

supernatant solution was decanted and the water used to wash the insoluble enamel was added to it. Aliquots of the combined solutions were analyzed for phosphorus. This served as a measure of the amount of decalcification occurring during the treatment with fluoride. Next, 10 ml. of 0.2 N acetic acid was added to the insoluble enamel. The mixture was shaken for 20 minutes as before. Following prompt centrifugation the supernatant solution was decanted and it was also analyzed for phosphorus.[6] Controls were run in the same manner except that fluorides were omitted.

The results are summarized in Table I. In this table is listed the amount of phosphorus liberated while the samples of enamel were being treated with the fluoride compounds. Also, data are given on the amount liberated by the subsequent treatment with dilute acetic acid. From the later data are computed the percentage decrease in solubility, as expressed in terms of phosphorus liberated, using as comparison values the control data obtained from enamel samples which were not treated with any kind of fluoride. Sodium fluoride was investigated in concentrations of 10, 500, and 9,050 ppm fluorine. The pH at each concentration was 2.5, 5.5 and 7.0. Stannous fluoride was tested similarly, except that the highest concentration of the latter was 4,840 ppm fluorine.

TABLE I

FLUORIDE	CONCENTRATION OF FLUORINE IN SAMPLE PPM	pH	NO. OF SAMPLES	P LIBERATED DURING PROTECTIVE TREATMENT MG./SAMPLE	P LIBERATED AFTER PROTECTIVE TREATMENT MG./SAMPLE	PERCENTAGE DECREASE IN SOLUBILITY
Sodium Fluoride	10	2.5	6	0.25	1.99	26
Sodium Fluoride	10	5.5	6	0.11	2.08	18
Sodium Fluoride	10	7.0	6	0.07	2.11	27
Sodium Fluoride	500	2.5	6	1.64	1.32	51
Sodium Fluoride	500	5.5	6	0.60	1.28	50
Sodium Fluoride	500	7.0	6	0.34	1.40	51
Sodium Fluoride	9,050	5.5	10	0.89	1.46	42
Sodium Fluoride	9,050	7.0	10	0.02	1.28	55
Stannous Fluoride*	10	2.5	12	0.12	1.73	36
Stannous Fluoride	10	5.5	12	<0.01	1.85	27
Stannous Fluoride	10	7.0	12	<0.01	1.72	40
Stannous Fluoride	500	2.5	12	0.50	0.31	89
Stannous Fluoride	500	5.5	20	0.12	<0.01	>98
Stannous Fluoride	500	7.0	6	<0.01	<0.01	>98
Stannous Fluoride	4,840	2.5	6	0.03	<0.01	>98
Stannous Fluoride	4,840	5.5	6	<0.01	<0.01	>98
Stannous Fluoride	4,840	7.0	4	<0.01	<0.01	>98
Water only	0.27	2.5	15	0.75	2.70	
Water only	0.27	5.5	14	0.55	2.53	
Water only	0.27	7.0	11	0.05	2.87	

*Prepared at Indiana University by Albert L. Hensley, Jr., to whom we are indebted.

Contrary to the conclusions of Phillips and Muhler[1] and of Palmer, Overstreet, and Sacks,[2] the data presented here show clearly that neither sodium fluoride nor stannous fluoride have any greater protective effect at a low pH than at higher pH values regardless of the concentration of fluoride used. Actually the degree of protection is shown to be substantially independent of the pH of the compounds used to protect the enamel. Thus the results agree with the clinical findings,[3] in that acidulated sodium fluoride is no more effective than neutral fluoride.

The protective effect of sodium fluoride at 500 ppm fluorine was decidedly greater than at 10 ppm fluorine, regardless of the pH, but at the level of 9,050 ppm fluorine (2 per cent sodium fluoride) the degree of protection was not significantly greater than at 500 ppm fluorine. This indicates that the latter concentration is near the maximum for the greatest protection which can be obtained with sodium fluoride. However, the maximum level for this compound allowed approximately one-half as much enamel phosphorus to be dissolved as in the case of enamel which was not treated with any source of fluorine.

Unlike sodium fluoride, stannous fluoride substantially prevented any enamel from dissolving when the concentration was 500 ppm fluorine or higher. This striking effect is in agreement with the evidence from other experiments[4-6] that this compound has great promise as an aid to dental caries prevention. The evidence indicates that it would have this marked action at any pH level ranging at least from 2.5 to 7.0. At the concentration of 500 ppm fluorine unbuffered stannous fluoride has a pH of approximately 2.9. At this pH it is readily soluble in water and solutions of it remain clear for several hours. When the pH is somewhat above 4 the solubility is much lower and precipitation of stannous hydroxide occurs more rapidly with time. Consequently, unbuffered stannous fluoride may be particularly advantageous in the effort to combat dental caries through topical application procedures. Through the cooperation of the Indiana State Board of Health, the compound is being tested for its effectiveness when applied topically to children.

SUMMARY

The effectiveness of sodium fluoride and of stannous fluoride in decreasing the solubility of powdered enamel is substantially independent of the pH at which the fluoride is applied to the enamel, as judged from experiments in which the pH ranged from 2.5 to 7.0. This is contrary to certain reports in the literature.[1, 2] The reason for the conflicting conclusions is presented.

In accord with other evidence cited, it is shown that stannous fluoride is much more effective than sodium fluoride in preventing powdered enamel from dissolving in acid.

REFERENCES

1. Phillips, R. W., and Muhler, J. C.: Solubility of Enamel as Affected by Fluorides of Varying pH, *J. D. Res.* **26**: 109, 1947.
2. Palmer, H. B., Overstreet, S., and Sacks, M.: An Evaluation of Various Techniques for Reducing Decalcification of Enamel. I. In Extracted, Erupted, Noncarious Third Molar Teeth, *J. D. Res.* **30**: 75, 1951.
3. Rickles, N. H., and Becks, H.: The Effects of an Acid and a Neutral Solution of Sodium Fluoride on the Incidence of Dental Caries in Young Adults, *J. D. Res.* **30**: 757, 1951.
4. Muhler, J. C., and Day, H. G.: Effects of Stannous Fluoride, Stannous Chloride and Sodium Fluoride on the Incidence of Dental Lesions in Rats Fed a Caries-producing Diet, *J. A. D. A.* **41**: 528, 1950.
5. Muhler, J. C., and Day, H. G.: Effects of Stannous Fluoride in the Food and in the Drinking Water on Caries Prevention in Rats on High Sucrose and Coarse Corn Diets, *J. Nutrition* **44**: 413, 1951.
6. Muhler, J. C., Boyd, T. M., and Van Huysen, G.: Effect of Fluorides and Other Compounds on the Solubility of Enamel, Dentin and Tricalciumphosphate in Dilute Acids, *J. D. Res.* **29**: 182, 1950.

MEASUREMENT OF DENTAL CARIES INCIDENCE IN SCHOOL CHILDREN USING A SODIUM BICARBONATE DENTIFRICE

ROBERT J. BRUCKNER, D.D.S., M.S., THOMAS J. HILL, D.D.S., AND BETTY J. WOLLPERT, B.S.

From the School of Dentistry, Western Reserve University, Cleveland, Ohio

IN VITRO studies of the effect of sodium bicarbonate[1] and the sodium ion[2] on the growth and acidogenesis of oral lactobacillus suggested the possibility that sodium might be of value in reducing the incidence of dental caries. The present investigation was initiated to determine the value of sodium bicarbonate as a dentifrice when employed by school children on a supervised toothbrushing program.

In considering methods for quantitatively estimating the extent of caries prevalence and incidence, difficulties that have received the serious attention of numerous investigators soon manifested themselves. Essentially, the problem may be stated as follows. When measuring caries experience in children, two variables must be considered, i.e., the susceptibility to caries, and the changing tooth population during the years of tooth eruption. At a given age the amount of tooth decay sustained by an individual is ultimately limited by the number of teeth or tooth areas available for caries attack. Therefore, some numerical consideration should be given to the variable and varying tooth population characteristic of young individuals. One approach to this problem has been that of employing the tooth or tooth surface population as the basis of measurement, and expressing caries experience as the per cent of teeth or surfaces that have been attacked.[3-6] Since teeth are susceptible to multiple caries attacks, the possibility exists that the use of "teeth" as a unit of measurement might be a less sensitive indicator than "tooth surfaces." As a consequence of these considerations, a second objective of this study was to make a comparison between several methods of caries measurement. These methods are (1) per cent of DMF teeth, (2) per cent of DMF surfaces, and (3) the number of lesions and fillings per 100 teeth.

MATERIAL AND METHODS

A dental examination was made for 548 school children 6 to 16 years of age who reside in a suburb of Cleveland. At the end of the one-year study 469 children were available for re-examination and it is the data for this latter group that are presented.

On the basis of mirror and explorer examination and bite-wing roentgenograms, each carious lesion and filling was recorded according to the surface or surfaces on which it occurred. Missing teeth and teeth with pulpal exposures, or near-exposures, due to caries were noted. Proximal lesions were

Supported in part by a joint grant from the Louis D. Beaumont Trust, the Cleveland Foundation, and the Elizabeth Severance Prentiss Foundation.

Received for publication, July 3, 1951.

recorded as involving one surface unless extension on the occlusal surface involved a caries-susceptible area, i.e., a pit or fissure. Proximo-occlusal fillings were recorded as involving the proximal and occlusal surfaces even though the occlusal extension may have been for the purpose of resistance form. Lesions and fillings were recorded individually. However, a surface on which decay was recurrent about the margins of a filling, or which contained a filling and a separate lesion was scored as one surface affected.

For each child a record of teeth present and teeth missing was obtained. Unerupted teeth were not counted as missing due to caries, of course. Partially erupted posterior teeth were considered present if a caries-susceptible area was visible clinically. Anterior teeth were considered present when a third or more of the crown was visible clinically. The tooth population for each child was determined as well as the number of tooth surfaces, each tooth considered as having 5 surfaces.

In recording the findings at the conclusion of the study, the scoring procedure was similar to the foregoing description. In addition, the number of carious and filled surfaces, lesions, and fillings on teeth which had been extracted during the course of the study were included in the final tally. Thus, in a given individual, all else being constant, the extraction of a carious or filled tooth could not result in an apparent lowering in the number of lesions and fillings or attacked surfaces. Likewise, a filling that restored two (or more) carious lesions was counted as two (or more) restorations. When, in making the final survey, the examiner failed to record a lesion reported in the initial survey, a re-examination of the tooth or teeth was made and the necessary correction inserted.

When dealing with DMF surfaces it is necessary to establish the value of M, i.e., the number of carious and filled surfaces on teeth missing because of caries. Values of 3,[7] approximately 4,[10] and 5[9] have been suggested. Hyatt[8] used the number of surfaces attacked on a tooth in the dental arch corresponding to the missing one. At the beginning of the present study M was determined by estimating the average number of carious and filled surfaces on teeth warranting extraction (teeth with exposures or near-exposures) and, hence, teeth that would be missing in the near future. For 122 posterior teeth this value was 2.7, for 6 anteriors, 1.5. At the end of the study the average number of carious and filled surfaces on 68 posterior teeth actually extracted during the year was 2.3. Only one anterior tooth had been lost and it had 1 surface involved.

For the purpose of studying the effectiveness of the dentifrice, a control and experimental group were selected at random, by choosing every third child on the classroom roster as a control. The only deviation from this procedure occurred when siblings were found to be in opposite categories. Inasmuch as rosters were not necessarily made up according to age, and because of subsequent nonparticipation by some pupils, changes of residence, illness, etc., the final ratio of experimentals to controls in each age group was altered from the intended 2:1 ratio.

Each school day, groups of pupils were taken to a washroom where they brushed their teeth under supervision of their teachers. Both teachers and pupils had been instructed in proper toothbrushing technique. It is known that difficulties were encountered in supervising the older groups of high school students so that the data for the age groups 14 to 16 years are of limited value. A sufficient quantity of dentifrice and toothbrushes was provided each pupil so that a home supply was available in addition to that maintained at school. Several educational movies related to maintenance of good oral hygiene were shown to the school children as well as to parents and teachers at P.T.A. meetings. Occasional letters were sent to the parents to stimulate and maintain their interest and cooperation, especially during vacation periods, with the object of promoting better home care of the teeth.

The dentifrice tested had the following formula: tricalcium phosphate, 40 Gm.; sodium bicarbonate, 60 Gm.; saccharin, .03 Gm.; methyl salicylate, 1.0 Gm. The control dentifrice consisted of tricalcium phosphate 100 Gm.; saccharin, .03 Gm.; and methyl salicylate .8 Gm. The dentifrices were dispensed in bottles each of which was labelled with a pupil's name and classroom number. In aqueous solution the bicarbonate dentifrice had a pH of 8.2 to 8.4, whereas that of the control was 6.9.

Data for lactobacillus counts on a total of 349 pupils were obtained on the basis of a salivary sample (collected upon rising in the morning) at the start and completion of the study.

The following abbreviations are used in this paper: DMFs refers to decayed, missing, and filled surfaces, DMFt refers to decayed, missing, and filled teeth, LF refers to lesions and fillings present. LMF includes lesions and fillings in teeth which had been extracted during the year of the study, in addition to the number of lesions and fillings present, T refers to total teeth available (erupted) and includes teeth missing because of caries, Tp refers to total teeth present. In the second survey (1951) it includes teeth present plus those lost because of caries during the 1950-1951 period, and S refers to total surfaces available, and includes surfaces of teeth missing because of caries. All data refer to permanent teeth only.

FINDINGS

Effectiveness of Dentifrice.—Because the supervision of the high school students was admittedly unsatisfactory in the upper grades, calculations were made which excluded ages 14 to 16, and these findings are presented in Table I. The per cent difference between the 1950 and 1951 findings or increment of caries attack, are tabulated for the experimental and control groups, respectively, as determined by the three methods of measurement. Thus, the 32.4 per cent difference between the 1951 and 1950 DMFs/S $\times$ 100 values for the experimental group compares with the 35.1 per cent difference for the control.

When the data are tallied to include the 14 to 16 year olds, the results shown in Table II are found.

TABLE I

THE PER CENT OF DMF TEETH, DMF SURFACES, AND THE NUMBER OF LESIONS AND FILLINGS PER 100 TEETH IN 419 SCHOOL CHILDREN 6 TO 13 YEARS OF AGE, INCLUSIVE, AT THE BEGINNING AND END OF THE TEST PERIOD

	$\frac{DMFt}{T} \times 100$		$\frac{DMFs}{S} \times 100$		$\frac{LF}{Tp} \times 100$	
	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL
1950	28.7	27.1	8.86 (8.93)	8.29 (8.33)	45.7	43.1
1951	37.6	36.3	13.10 (13.15)	12.78 (12.81)	67.9*	66.8*
Difference	8.9	9.2	4.24 (4.22)	4.49 (4.48)	22.2	23.7
% Difference	23.7	25.3	32.4 (32.1)	35.1 (35.0)	32.7	35.5

Two hundred eighty-three in the experimental group, 136 in the control. Values in parentheses are those obtained when M equals 2.7.

*These values are LMF/Tp × 100.

In order to evaluate the bacterial counts, a distribution of the per cent of children, 6 to 13 years old, with counts above and below 10,000, respectively, was calculated for the experimental and control groups (Table III). Only 9 samples of saliva were obtainable in the 14 to 16 age groups and these are not included in the data shown. The per cent of children with counts below 10,000 decreased from 39.1 to 37.9 in the experimental group and from 35.1 to 33.3 in the control. The per cent of children with counts above 10,000 increased from 60.9 to 62.1 in the experimental group and from 64.9 to 66.7 in the control.

Comparison of Methods.—The yearly increment of caries attack in the experimental and control groups as measured in terms of per cent teeth and per cent surfaces that are DMF, as well as by the number of lesions and fillings per 100 teeth is shown in Tables I and II. For the experimental group, ages 6 to 13 only, the per cent difference between the 1950 and 1951 values are 23.7, 32.4, and 32.7, depending on the method of calculation. For the control group the corresponding values are 25.3, 35.1, and 35.5. Similar calculations for age groups 6 to 16 are shown in Table II.

TABLE II

THE PER CENT OF DMF TEETH, DMF SURFACES, AND THE NUMBER OF LESIONS AND FILLINGS PER 100 TEETH IN 469 SCHOOL CHILDREN 6 TO 16 YEARS OF AGE, INCLUSIVE, AT THE BEGINNING AND END OF THE TEST PERIOD

	$\frac{DMFt}{T} \times 100$		$\frac{DMFs}{S} \times 100$		$\frac{LF}{Tp} \times 100$	
	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL
1950	31.1	31.1	9.77 (9.86)	9.82 (9.92)	48.7	47.6
1951	39.6	39.9	14.00 (14.07)	14.16 (14.25)	70.7*	70.9*
Difference	8.5	8.8	4.23 (4.21)	4.34 (4.33)	22.0	23.3
% Difference	21.5	22.1	30.2 (29.9)	30.6 (30.4)	31.1	32.9

Three hundred ten in the experimental group, 159 in the control. Values in parentheses are those obtained when M equals 2.7.

*These values are LMF/Tp × 100.

DISCUSSION

The Dentifrice.—Sodium bicarbonate has been used empirically as a dentifrice for many years, either alone or in combination with other substances. Laboratory studies[1, 2] suggested that sodium bicarbonate or the sodium ion are inimical to the growth and acidogenicity of oral lactobacillus. While the relationship of specific bacteria to the etiology of dental caries remains a moot question, the possibility that sodium bicarbonate, a relatively inexpensive household article, might exert an effect on these organisms in vivo, offered an attractive approach to potential dental caries control. In addition, were this salt to inhibit the lactobacillus flora in vivo as well as in vitro, any accompanying change in caries activity or any failure to obtain a change would be a further contribution to our knowledge of the caries mechanism. These two objectives, therefore, motivated the use of this dentifrice.

A slightly lower caries increment can be found in the experimental group as compared with the control, irrespective of the method of measurement used (Tables I and II). The difference between the group using the bicarbonate dentifrice and the one using the control dentifrice can not be considered clinically significant and is probably due to fortuitous sampling variation entirely.

It has been shown that brushing the teeth immediately after eating is an effective method in reducing caries incidence.[11] In the present study the supervised program was so arranged, of necessity, that brushing of the teeth was carried out during morning classes and did not immediately succeed the morning or noon meal. Home care of the teeth, despite its encouragement, could not be depended upon to be carried out faithfully, but the possibility existed that some home care, plus the daily supervised introduction of an alkaline sodium salt into the oral cavity, might exert a beneficial effect whether used after eating or not. One effect that could be independent of the time of day the teeth were brushed, was that of the alkaline dentifrice on lactobacillus counts.

TABLE III

THE PER CENT OF CHILDREN WITH LACTOBACILLUS COUNTS ABOVE OR BELOW 10,000 PER C.C. OF SALIVA AT THE BEGINNING AND END OF THE TEST PERIOD

	EXPERIMENTAL		CONTROL	
	1950	1951	1950	1951
Below 10,000	39.1	37.9	35.1	33.3
Above 10,000	60.9	62.1	64.9	66.7

Saliva samples were obtained from 235 in the experimental group, 114 in the control.

As indicated in Table III, both the experimental and control groups showed similar changes in frequency distribution of pupils with bacterial counts above or below 10,000. The changes observed between the initial and final values for the experimental and control groups are so subtle that one hesitates to place a literal interpretation on the differences. The observation might be made, however, that these two groups of individuals with similar caries incidence also presented comparable indices of caries activity as determined by lactobacillus counts. Whether or not this observed relationship is

circumstantial, touches upon one of the basic problems concerned with the etiology of dental caries. Nevertheless, the two groups of children presented a consistent and comparable history of caries attack whether based on direct measurement in the oral cavity or the indirect method based on bacterial counts.

Methods of Measurement.—As stated in the opening paragraphs, the "sensitivity" to alterations in caries attack using "teeth" as the basis of measurement, was a point to be investigated. In the situation that finally developed, the two groups of children were very much alike in respect to caries incidence, and this fact could be ascertained by any one of the three methods used (see Table I). This similarity between the two groups limits the evaluation of the advantages, if any, that any one method may have been used to compare caries incidence in relatively small groups of individuals. It cannot be ascertained from the data whether or not all three measuring methods would give comparable results when used to evaluate groups with divergent caries incidence.

In connection with the study of the qualities of the three methods used, the following observation was made. When comparing the relative increase in caries prevalence for the entire group of individuals as measured by the three methods, the use of per cent DMFs and L(M)F per 100 teeth gave similar results. That is, the 1951 values are a similar number of times higher than the 1950 values when these two methods are used. (See parallel slope of lines representing combined data for experimental and control groups, Fig. 1). Also compare per cent difference in Table IV.) The use of per cent DMFt

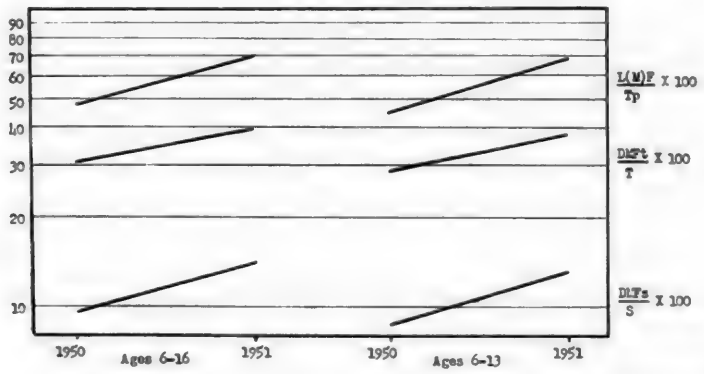

Fig. 1.—Graph showing the relative increase in caries prevalence for the experimental and control groups combined as measured by the three methods of calculation indicated. Data are plotted for the entire group of school children studied as well as for ages 6 to 13 inclusive.

gives a slope of lesser inclination than that of the other two, an observation which may be attributed to the fact that multiple caries attacks are somewhat obscured when per cent DMFt is employed.

The use of M equals 2.7 would have had little effect on the conclusions drawn. Having determined the observed average of 2.3 carious or filled sur-

TABLE IV

THE PER CENT OF DMF TEETH, DMF SURFACES, AND THE NUMBER OF LESIONS AND FILLINGS PER 100 TEETH IN 469 SCHOOL CHILDREN 6 TO 16 YEARS OF AGE, AND 419 SCHOOL CHILDREN 6 TO 13 YEARS OF AGE AT THE BEGINNING AND END OF THE TEST PERIOD

		$\frac{DMFt}{T} \times 100$	$\frac{DMFs}{S} \times 100$	$\frac{LF}{Tp} \times 100$
Ages 6-16	1950	31.1	9.79	48.3
	1951	39.7	14.05	70.8*
	% Difference	21.7	30.3	31.8
Ages 6-13	1950	28.2	8.70	44.8
	1951	37.2	13.00	67.5*
	% Difference	24.2	33.1	33.8

*These values are LMF/Tp × 100.

faces per tooth actually extracted, the 2.7 value becomes a rather academic one. Yet, it serves to accentuate the innumerable variables that complicate the task of quantitative caries measurement. For example, in some individuals, teeth that were cariously exposed and unquestionably warranted extraction at the start of the study were still present a year later because there had been no painful or untoward symptoms of an acute nature. This somewhat disconcerting finding contributed to the discrepancy between the original estimate of 2.7 and the observed value of 2.3. For this and other reasons, the latter value applies to this group of children only. It is interesting to note that the value obtained is lower than those estimated by other investigators on the basis of general observation[7] or studies of extracted teeth.[10]

A few remarks should be made concerning the use of the ratio L(M)F/Tp. The original (1950) survey measures the number of lesions and fillings found per 100 teeth present in the oral cavity of the individuals studied. This establishes a base line for the second survey and does not attempt to measure total caries experience in the sense that teeth missing because of caries are included. The second survey (1951) includes the number of lesions and fillings found, plus those lesions and fillings which were present in teeth extracted during the 1950-1951 period. Included, too, are the teeth present, plus those extracted during the period of the study. In other words, the ratio is derived from lesions, fillings, and teeth observed at the time of and subsequent to the initial examination.

CONCLUSIONS

1. The use of sodium bicarbonate as a dentifrice for approximately one year lowered the incidence of dental caries in a group of children very slightly when compared to a control group using a neutral dentifrice. The difference between the two groups was not considered clinically significant.

2. When comparing two groups of children with a *similar* caries incidence, the use of per cent DMFt,* per cent DMFs,* or the number of lesions and fillings per 100 teeth each yielded the same information, viz., both groups sustained a similar caries attack during the course of the study with a slightly lower incidence observed in the experimental group than in the control.

*For key to abbreviation, see page 107.

3. The slight change in frequency distribution of the per cent of children with lactobacillus counts above or below 10,000 was comparable in the experimental and control groups. The similarity of the bacterial findings for the group using the bicarbonate dentifrice and the control group was consistent with the clinical observations.

4. The use of the ratios DMFs/S and L(M)F/Tp gave comparable estimates of caries incidence. The use of the ratio DMFt/T gave an estimate of smaller dimension than the other two and this was attributed to the fact that multiple caries attacks are partially obscured when DMF teeth are employed as the basis of measurement.

5. For this group of children, during the period of the study, there was an average of 2.3 carious or filled surfaces per extracted (missing) tooth.

The authors wish to acknowledge the cooperation given them by Mr. Gerald H. Michel, Superintendent of the Wickliffe Schools, Mrs. Nora Stoneman, Principal of the Lincoln School, and their teachers. The generous assistance of Mrs. Jessie Jewett, R.N., is particularly appreciated. Lt. John K. Dunn, USN, former dentist for the Wickliffe Schools, contributed time and effort to the initial phases of the study, for which the authors are appreciative. Assistance in taking and processing radiographs was kindly provided by Drs. Milton Gellin, Morris Matt, and Robert Porter.

For advice, suggestions, and constructive criticisms in regard to problems concerned with the measurement of caries attack, the authors are indebted to Dr. George F. Badger.

REFERENCES

1. Bruckner, R. J.: In Vitro Studies of Sodium Bicarbonate in Relation to Certain Factors Concerned with Dental Caries, *J. D. Res.* **27**: 128, 1948.
2. Pearlman, S.: The Action of Ammonia Salts on Lactobacillus Acidophilus (4646), *J. D. Res.* **28**: 638, 1949.
3. Bibby, B. G.: A Test of the Effect of Fluoride-Containing Dentifrices on Dental Caries, *J. D. Res.* **24**: 297, 1945.
4. Day, C. D. M., and Sedwick, H. J.: Studies on the Incidence of Dental Caries, *D. Cosmos* **77**: 442, 1935.
5. East, B. R., and Pohlen, K.: Health Index of Teeth, *J. D. Res.* **20**: 533, 1941.
6. Sloman, E.: Sex and Age Factors in the Incidence of Dental Caries, *J. A. D. A.* **28**: 441, 1941.
7. Bödecker, C. F., and Bödecker, H. W. C.: A Practical Index of the Varying Susceptibility to Dental Caries in Man, *D. Cosmos* **73**: 707, 1931.
8. Hyatt, T. P.: How Dental Statistics Are Secured in the Metropolitan Life Insurance Company, *J. D. Res.* **9**: 411, 1929.
9. Klein, H., and Palmer, C. E.: The Prevalence and Incidence of Dental Caries Experience, Dental Care, and Carious Defects Requiring Treatment in High School Children, *Public Health Reports* **55**: 1258, 1940.
10. Wessels, K. E., and Cheyne, V. D.: A Determination of the Number of Surfaces Involved in Carious Extracted Teeth, *J. D. Res.* **26**: 375, 1947.
11. Fosdick, L. S.: The Reduction of the Incidence of Dental Caries. I. Immediate Tooth Brushing with a Neutral Dentifrice, *J. A. D. A.* **40**: 133, 1950.

A MARK SENSE CARD FOR RECORDING DENTAL EXAMINATION FINDINGS

ELLIOTT H. PENNELL AND SAMUEL S. HERMAN

Division of Dental Resources, United States Public Health Service, Washington, D. C.

THE statistical management of mass data presents recording and tabulating problems which frequently can be solved most efficiently through use of punch cards and mechanical equipment. From time to time development of new equipment and improvements or additional features for existing units have permitted simplification of operations with an attendant gain in speed and accuracy. One such advance has been the development of mark sensing, a procedure initially used in administering multiple choice examinations to allow rapid mechanical scoring of entries. This procedure has been extended to the automatic punching of tabulating cards on which information has been marked with an electrographic pen or pencil.

THE MARK SENSING PRINCIPLE

A card designed for mark sensing is laid out in a fixed pattern of 27 columns which may be arranged in conventional fields. This pattern may be repeated on the back of the card to permit the marking of a total of 54 columns of information.

For punching purposes, the cards are passed through a reproducer equipped with a mark sensing device, which "senses" or "reads" the marks and makes a perforation in the card corresponding to the relative position of each mark. Any of the 27 mark sensing columns on either side of the card can be "read" and punched into any of the standard 80 punching columns. Before the card is ejected it may be mechanically scanned, column for column, to insure the presence of one and only one hole in the columns selected for punching. Since the "punching and verification" are accomplished mechanically through the marking of the original record, the punched-card routine, from recording of the basic data to the preparation of tabular material, becomes completely automatic.

DESCRIPTION OF A MARK SENSE DENTAL RECORD FORM

An application of the mark sensing technique to the recording of dental examination findings was reported some years ago by Klein and Kramer,[1] who developed a card for tabulation of the caries experience of the individual teeth in both dentitions. A later test of this form by Klein[1] in his survey of a Nova Scotia population group established its utility as an instrument for the recording and analysis of detailed caries epidemiologic data.

The present communication describes a further application of the mark sense card in which the principle focus is on the accumulation of a series of summary statistics descriptive of the dental health status of groups of individuals. The present form is designed to provide inexpensively and quickly those tabulated totals which experience suggests are of most frequent concern to the program administrator for planning, evaluation, and study purposes.

Received for publication, Oct. 6, 1951.

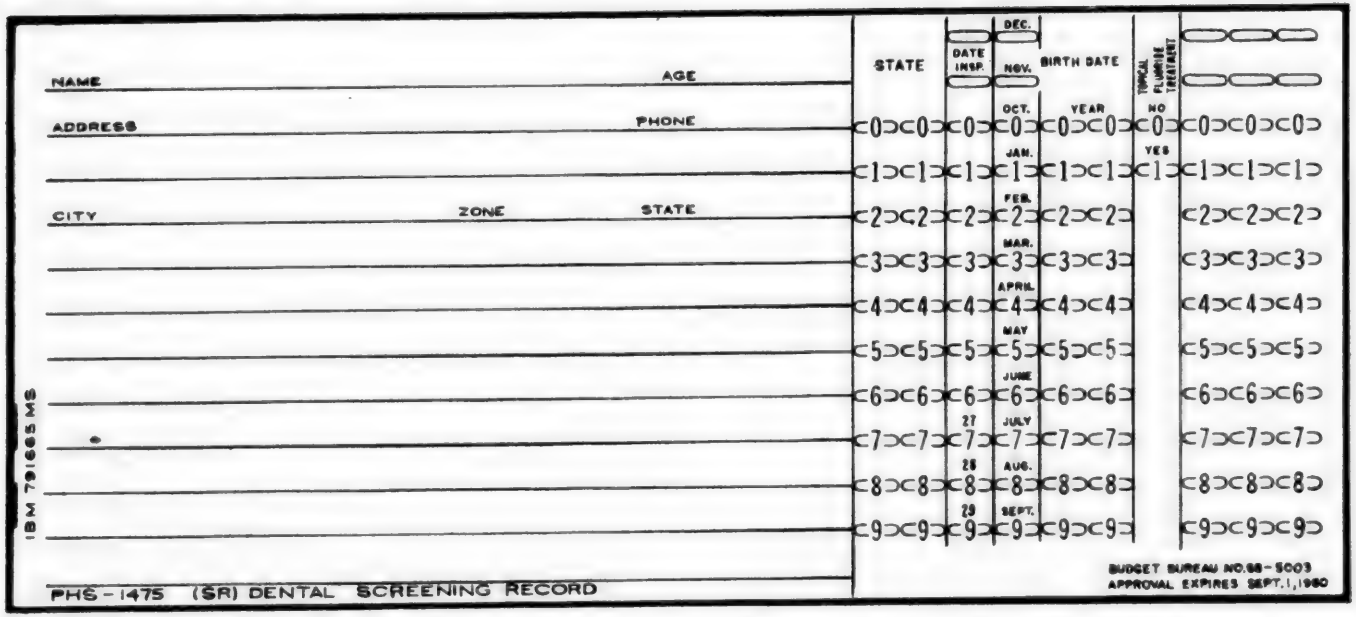

Fig. 1.—Front of mark sense record form (to be completed by recorder preliminary to dental examination).

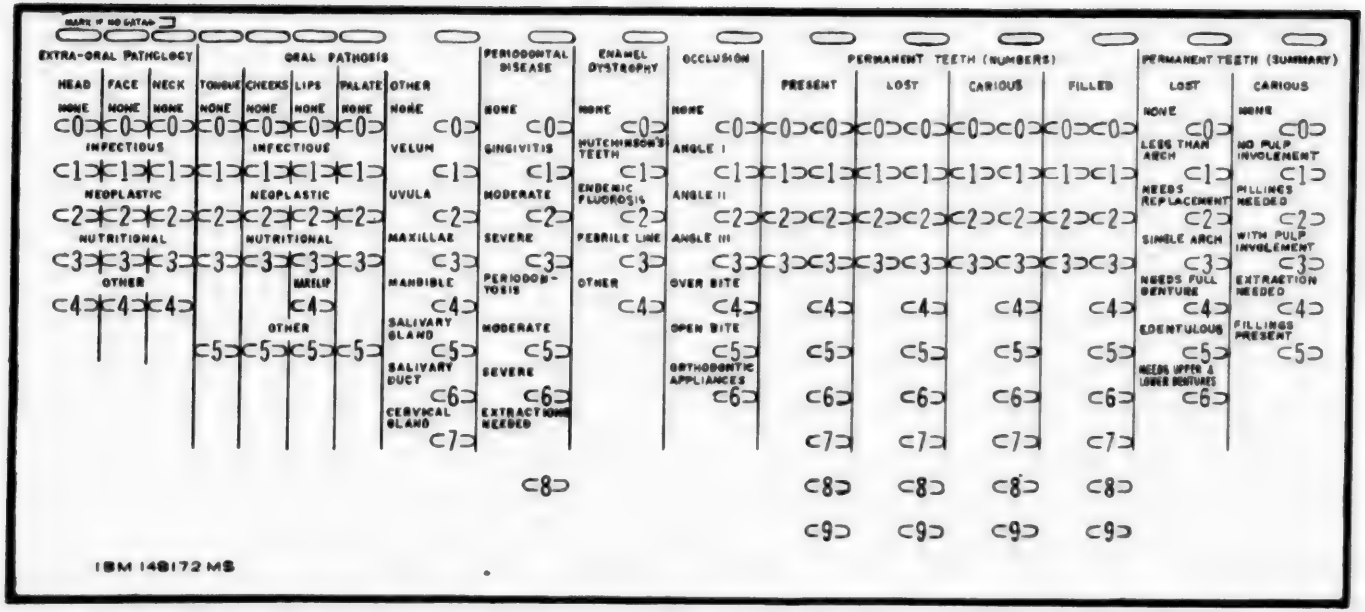

Fig. 2.—Back of mark sense record form (to be completed by recorder from information dictated by examining dentist).

For example, the public health dentist customarily requires knowledge of the frequency of decayed, missing, and filled teeth in a population group of a particular age composition. Previous record systems for the collection of these data necessitated intermediate transcription and coding of summary figures and in most cases a subsequent key punching and verification. The present procedure eliminates the need for assembling, coding, or key punching of data after the examination is performed and recorded.

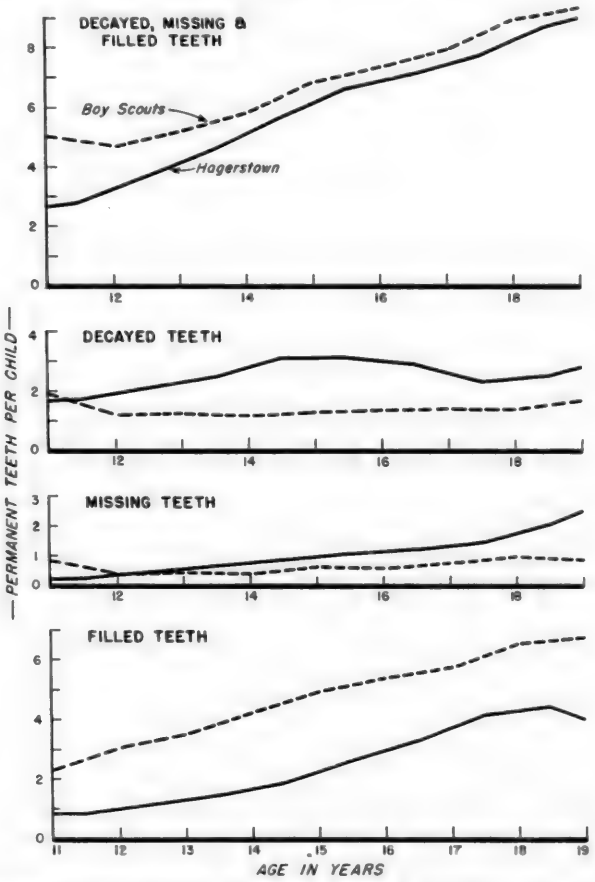

Fig. 3.—Age-specific caries experience rates of Boy Scouts examined at 1950 Jamboree and of school children examined in Hagerstown, Md., 1937.

The face of the card (Fig. 1) is laid out in two sections for the recording of identifying material. In the first section, name, age, address, telephone, etc., are entered in longhand as descriptive data. Extra lines are provided for recording clinical comments and miscellaneous observations. The mark sense section is divided into several fields for entering the State code, date of inspection, year and month of birth, and receipt of topical fluoride applications. Three marking columns are left for the recording of additional characteristics. For example, this field may be used for identification of the examining dentist.

The back of the card (Fig. 2) is arranged for entering dental examination findings in the following seven fields: (1) extraoral pathology—absence or presence of infections, neoplastic, nutritional, or other lesions, according to site (head, face or neck); (2) oral pathosis—absence or presence of intraoral disorders, by site of occurrence (tongue, palate, uvula, etc.); (3) periodontal disease—absence or presence of gingivitis or periodontosis on a basis of severity; (4) enamel dystrophy—structural imperfections, such as Hutchinson's teeth, fluorosis, and febrile line; (5) occlusion—absence or presence of malocclusion (Angle); (6) permanent teeth (numbers)—totals of permanent teeth present, lost, carious, or filled; and, (7) permanent teeth (summary)—summary of lost permanent teeth in terms of treatment needs and of carious permanent teeth in terms of severity of attack.

FIELD TESTING OF RECORDED FORM

The selection of examination items in the present card parallels somewhat the pattern of dental information recorded in the Greater Atlanta Health Survey, conducted by the Georgia Department of Public Health in cooperation with the U. S. Public Health Service.[2] The utility of the form was tested in a health survey of over 7,000 boys who attended the 1950 National Boy Scout Jamboree at Valley Forge, Pennsylvania.

Identifying information was entered on the face of the record by a clerk, preliminary to the dental examination. The inspection technique employed in this project was of a screening type in which the examining dentists used a tongue depressor and a flashlight. The examiner dictated his observations in the sequence indicated on the card and a clerk recorded the information in the appropriate mark sense field. The entire examining-recording operation averaged less than two minutes per individual.

Machine processing of the data provided a detailed breakdown of the examination findings according to age and geographic region. From this material it was relatively simple to abstract summary figures useful for administrative study. For example, age-specific caries experience rates (DMF) derived from the Valley Forge screening project were compared with those of the earlier Hagerstown studies by Klein and Palmer.[3] Fig. 3 discloses a uniformly greater caries experience rate (DMF) for all ages of the Boy Scouts, resulting largely from the higher frequency of filled teeth in this group. On the other hand, the Hagerstown rates for missing and decayed teeth exceed those of the Boy Scout series at all ages. The higher filled tooth rates observed at the Jamboree probably reflect both the heightened health consciousness fostered in these boys by the scouting movement and the last minute efforts of the boys to have all remediable defects corrected for the Jamboree. The lower missing tooth rates observed in the Boy Scouts are consistent with the Hagerstown findings of decreased tooth loss in children with fillings as compared to children without previous fillings.[4] These data further document the effectiveness of dental care in the reduction of tooth mortality.

DISCUSSION

The principal advantage of the mark sense record form described above derives from the reduction in clerical and punching labor made possible by its use. Information is obtained at the source and the "punching" is in effect performed on the job. In cases where repetitive data such as place and date of examination are to be entered on a large number of records, partially prepunched and interpreted cards may be used. The cards are then completed by marking the remaining information required thereby effecting a savings in clerical time per examination.

The punching of the 7,239 completed records from the Boy Scout screening project was accomplished in less than two ordinary work days by passing the cards through an automatic reproducer. As indicated earlier, the mark sense card serves also as the original record, thereby eliminating the need for further verifying. Completion of the basic material for analysis required three to four runs through the tabulator, which were performed in 1½ days.

A disadvantage in the use of a mark sense record lies in the need for greater than ordinary care in physical handling since these cards must pass through statistical machines. Proper instruction of clerical personnel is also required to insure efficient marking performance.

Despite these disadvantages, experience in the use of this form has encouraged the Division of Dental Resources to study further use of field marked cards for the assembling of administrative statistics. At the present time an experimental record form has been developed for reporting the accumulated dental needs of Public Health Service beneficiaries. Design and utilization of this record will be described in a forthcoming report.

REFERENCES

1. Klein, H., and Kramer, M.: A Dental Examination Record Form for Statistical Machine Tabulation, *J. D. Res.* **27**: 170, 1948.
2. Bowdoin, C. D.: The Georgia Plan of Multiphase Testing, *Southern Med. J.* **44**: 236, 1951.
3. Klein, H., and Palmer, C. E.: The Disparity Between Dental Need and Dental Care in School Children of Hagerstown, Maryland, and Environs, *J. A. D. A.* **28**: 1489, 1941.
4. Knutson, J. W., Klein, H., and Palmer, C. E.: Dental Needs of Grade School Children of Hagerstown, Maryland, *J. A. D. A.* **27**: 579, 1940.

DEPTH TESTER FOR DENTAL CARIES LESIONS

CHARLES F. BODECKER, D.D.S.*

Center Moriches, N. Y.

THE measure of the efficacy of an optimal diet or of fluorine therapy in reducing dental caries is dependent on a precise determination of the activity of the caries at the beginning and at the end of the observation period. The usual technique for measuring this efficacy is the careful charting of the extent of carious tooth destruction. Two of the commonly used methods are the DMF Index (decayed, missing, and filled teeth) of Klein and Palmer[1] and the Modified Dental Caries Index by surfaces (Bodecker[2, 3]).

Even though these two methods are reliable in defining the rate of carious destruction of the teeth, they do not give information concerning the extant and character of the destructive changes within the carious lesion. Data concerning cavity depth and, particularly, the texture of carious dentin at the base of the cavity are important in judging the immediate efficacy of experimental control methods. Nutritional studies have been reported in which the observers have noted a hardening of the carious dentin at the base of cavities after placing the subject on an optimal diet. However, those investigators who minimize the effect of diet in raising the resistance of the teeth against caries, do not accept this subjective observation without more definite proof.

The problem of the manner in which dentin becomes hard, as observed in arrested caries, shows the need of an instrument which accurately measures cavity depths at various time intervals during the experimental periods. Such an instrument may be useful in settling this long contested problem. Is the hardness of the floor of a cavity noted in arrested caries due to: (1) elimination of the decalcified dentin by the force of mastication, or (2) is the decalcified dentin hardened by salivary salts or by other means? If the latter is the case, subsequent measurements should show a decrease in cavity depth.

Three requisites of an effective cavity depth tester for this purpose appear to be: (1) It must determine the exact depth of a carious cavity; (2) it must have the capacity for repeating measurements of an identical spot in a carious cavity; and (3) it must indicate possible changes in the physical character of the carious dentin at the base of the cavity.

An instrument fulfilling these requisites is herewith described. It was designed through the activities of the Columbia University Dental School Caries Research Group and it was built by David Crowther, the machinist of that school. Fig. 1 is a drawing of the cavity depth testing instrument. A fine pointed needle, movable in vertical directions (arrows), is retained in a holder. The movement of the needle is controlled by a spring (S), pressing on a ball

*Professor Emeritus, Columbia University, School of Dental and Oral Surgery of the Faculty of Medicine, New York, N. Y.; Adviser in Research, Dental Department, Montefiore Hospital, New York, N. Y.

Received for publication, Aug. 26, 1951.

(B), which maintains it under uniform pressure. By screwing the ferrule (F) in or out, the friction on the needle may be varied, making it slide more or less easily. A calibrated scale (CS) shows the position of the ferrule and thus assures the establishment of the same spring pressure used in a previous observation.

The depth measurement of a carious lesion is taken by inserting the needle into the cavity and pressing the holder until it comes in contact with the occlusal surface of the carious tooth. The friction of the needle causes it to penetrate the soft, decalcified dentin under a pressure of 110 to 120 Gm., retaining it in its position in the holder for subsequently determining the depth of the cavity.

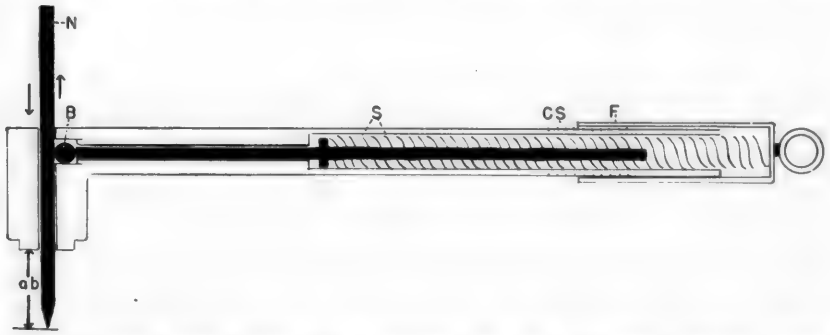

Fig. 1.—Holder with needle (N) to measure the depth of carious cavities. Ball (B) under uniform pressure of spring (S) presses against needle which is movable in two directions (arrows). The depth of cavity is equal to "a-b." Screwing down ferrule (F) increases pressure on needle, making it move less easily and therefore needle penetrates decalcified dentin more readily. CS, calibrated scale to show position of ferrule.

The next problem in the development of the instrument was to measure exactly the distance "a-b" (Fig. 1) which represents the depth of the cavity. This offered some initial difficulty. The use of a standard micrometer was not feasible, because it was difficult to determine exactly the moment when contact had been made between the point of the needle and the jaws of the instrument. To avoid this difficulty, any one of the 3 following methods may be used to retain the needle in its position while measuring the distance "a-b" (Fig. 1): (1) A set screw in the head of the holder could immovably fix the needle after the cavity depth measurement has been taken; (2) measuring might be done under a low-powered lens with a magnification of approximately 10×. The operator could then view the approach of the micrometer jaw to the needle point more clearly and thus prevent the former from forcing the needle into the holder; and (3) an electrical circuit could be installed so that a light would flash at the moment of contact of the needle point with the micrometer. Mr. Crowther, the machinist, suggested this method, which proved satisfactory.

After a cavity measurement has been obtained, the holder is hung in the apparatus (Fig. 2). The distal end of the needle passes into a slot (S) drilled into the fixed jaw of the micrometer, so as to leave the position of the needle in the holder undisturbed. When the holder is in this position, its distal surface is in contact with the support and establishes one electrical contact. The

micrometer is then closed cautiously, so as to approach very slowly the point of the needle. The moment contact occurs, the electrical circuit is closed and a light flashes. The electric bulb (B) and dry cell (C), furnishing the current, are seen in Fig. 2.

The cavity depth tester was never used for a project by any member of the Research Group because accurate duplication of measurement could not be achieved at that time. The reason for the variability of the measurements was that the needle point could not again be placed on the identical spot in the base of the carious cavity which had originally been contacted. This difficulty has now been overcome by the following procedure: A piece of thin copper plate (CP, Fig. 3), commonly used in taking impressions for full veneer crowns, is cut to a size slightly smaller than the occlusal surface of the carious tooth. The copper is heated and a small amount of very hot impression or modelling compound is added on one surface and this is pressed against the occlusal surface of the tooth, allowing some of the compound (MC) to enter the cavity. The impression is chilled and removed, a hole is drilled at right angles to the copper

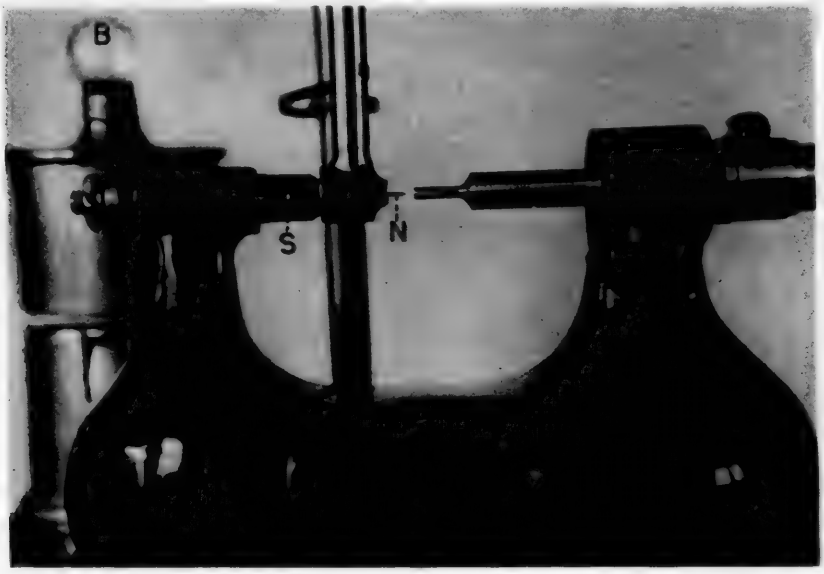

Fig. 2.—Close-up of a part of measuring apparatus which determines cavity depth (a-b of Fig. 1). S, slot into which the distal end of the needle fits; B, bulb flashes when point of needle (N) makes contact; C, dry cell.

plate in the area, corresponding to the approximate center of the cavity, with a bur of exactly the same diameter as the cavity depth testing needle. The above procedure results in an impression of the orifice of the cavity and the entire occlusal surface of the tooth, so that the testing needle (N), passing through the hole in the copper plate, always reaches the same spot at the base of the cavity during subsequent measurements. Employing this method, duplicatable measurement with variations of 8 to 20 microns were made possible. This

margin of error is insignificant, as increases in depth due to caries of 400 or more microns have been observed in subsequent measurements of the same cavity.

The most suitable cavities for measurment are those involving the occlusal surfaces of molars and premolars. Here the copper reinforced modelling compound impression finds a firm and exact bearing surface, so that it always assumes its original position. One precaution must be taken; this is to drill the hole as nearly as possible at right angles to the copper plate. Inaccurate subse-

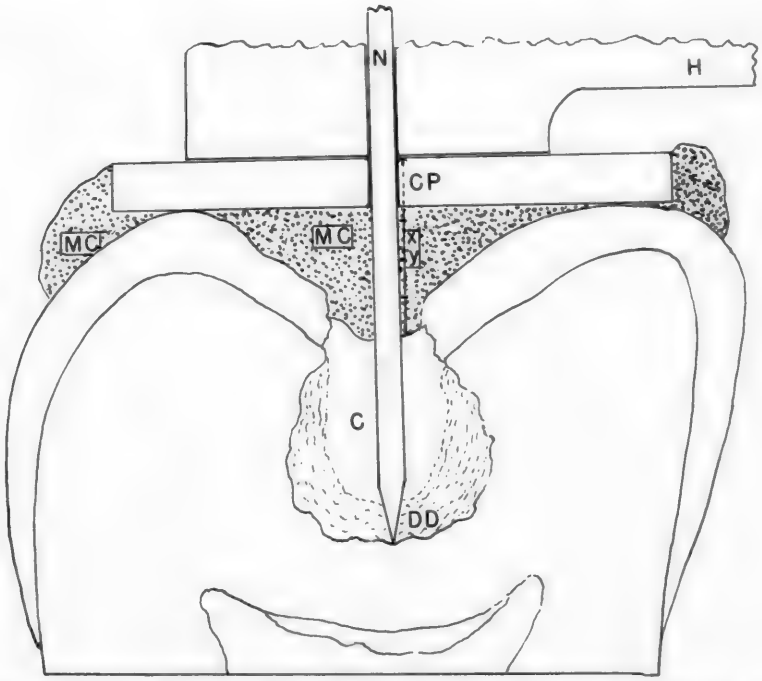

Fig. 3.—Method of measuring depth of carious cavities assuring duplicatable measurements. H, part of holder for measuring needle (N) which passes through hole in copper plate (CP) and modelling compound (MC); x-y, length of bearing for needle, should be as long as possible to secure duplicatable measurements; C, carious cavity in occlusal surface of molar. Hardness of decalcified dentin (DD) is tested by sharply pointed needle. If this area is subsequently hardened by salivary salts as a result of change in diet, etc., needle will not penetrate as deeply as on first examination, thus showing an increase in cavity floor hardness.

quent measurements result, if this precaution is not heeded, because if the hole is drilled at an angle, the face of the holder (Fig. 3) will not rest squarely on the copper plate (CP). In such case, only slight deviations of the handle to the right or left result in subsequently inaccurate measurements.

The depth of cavities on the cervical areas of teeth may also be measured, but not with the same degree of accuracy as those on the occlusal surface of molars and premolars, because cervical areas offer a less firm and extensive foundation for the compound impression than that furnished by the occlusal

surface of a molar or premolar. However, by festooning the copper plate so that it does not encroach upon the resilient gingival tissue, an impression can be made which rests fairly firmly on the labial or buccal surface of a tooth.

A few further suggestions may be added. Only a small amount of modelling compound should be used in order to prevent the impression being held in the cavity and distortion on removal as a result of the undercuts which usually are present in molars. However, as much modelling compound as possible should be used so that the needle has thorough guidance. The reason for this is made clear in Fig. 3. The bearing surface for the needle's (Fig. 3, x-y) guiding to the base of the cavity is composed of modelling compound (MC) and the orifice of the copper plate. The longer this "bearing" is, the more exactly can subsequent measurements be taken. Short "bearings" permit the needle point to swing in a small arc, thus contacting different spots in the cavity base and resulting in successively different measurements.

Retention of the impression as a result of overhanging enamel margins can be obviated by previously removing them with a chisel, but opening the cavity makes it less food retentive which, in itself, may result in a reduction of caries activity. Hence, the breaking down of enamel margins for the purpose of obtaining a better impression may lead to incorrect conclusions regarding observations on the effects of caries preventive measures, such as the effect of diet or of fluorine therapy.

The instrument should be sterilized in an oil bath to prevent the rusting of the steel spring and ball within the holder.

SUMMARY

An instrument has been constructed which affords a means of determining slight variations in the progress of dental caries. Its purpose is twofold: (1) To obtain reproducible measurements of the depth of carious cavities, thus determining the rate of progress of the disease; (2) to determine possible variations in the hardness of the decalcified dentin on the floors of carious cavities.

Its use should give determinative information on the effects of caries control procedures: (a) If beneficial, the depth of the cavity will increase more slowly than in controls at successive measurements; if the protection is complete, the depth of the cavity will remain the same. (b) If caries is arrested, light may be thrown on the following controversial question: Is the hard floor of cavities noted in arrested caries composed of previously decalcified dentin which has been recalcified by salivary salts or by other means, or has the previously decalcified dentin been eliminated by the force of mastication? In the former case, subsequent measurements should show a decrease in cavity depth; in the latter, an increase in cavity depth.

REFERENCES

1. Klein, Henry, and Palmer, C. E.: *Pub. Health Bull.* No. 239, Washington, D. C., 1938, Government Printing Office.
2. Bodecker, C. F.: *D. Survey* **7**: 23 and 59, 1931.
3. Bodecker, C. F.: *J. A. D. A.* **26**: 1453, 1939.

EPIDEMIOLOGIC STUDIES IN DENTAL CARIES

IV. Variability of Progression Rates of Dental Cavities in the Occlusal Surfaces of Second Molar Teeth

JULIAN D. BOYD, M.D., KENNETH E. WESSELS, D.D.S., AND
ROBERT E. LEIGHTON, D.D.S.

From the Department of Pediatrics, College of Medicine and the Department of Pedodontics, College of Dentistry, State University of Iowa, Iowa City

MOST dentists have observed that some dental cavities progress rapidly in size, whereas other cavities may show but slight tendency toward progression. Dental literature does not provide sufficient information relating to the time factor in caries evolution, especially as to the variability one may expect to observe among different dental cavities. Under the usual conditions of practice, the dental operator will tend to place fillings in cavities as soon as decay becomes evident. This procedure may lead one to assume that it is unsafe to leave the average cavity untreated for any prolonged period of time. Once such a cavity has been filled, there is no way that one can judge whether without therapy that specific cavity would have progressed rapidly, or whether it might have remained latent for months or, possibly, for years.

It would be helpful for the practicing dentist if he had tables of predictability as to rates of progression of tooth morbidity. For the dental investigator even more than for the practitioner, knowledge should be available as to the spontaneous variability of caries progression rates which one may expect to observe among untreated cavities in a general population. The observer then would be less likely to confuse such normal deviations from the group averages with those deviations which he might properly attribute to the action of any agent whose caries-potentiating effect he might be testing.

In the course of a long-term study of dental findings within a specific child population, we have accumulated serial records of the status of individual teeth of more than two hundred children throughout several successive years.[1, 2] These children received only emergency dental therapy while under observation, and as a consequence it has been possible to analyze the serial course of each untreated cavity. Each dental record portrayed the size, location and relative depth of every cavity, as well as the consistency of the adjacent tooth substance as gauged by its resistance to the exploring tine. The dental examinations were made in a painstaking manner by specially trained dentists, using sharp exploring tines, mouth mirrors and adequate illumination. Bite-wing radiographs of the posterior teeth were made routinely, and their findings were used to supplement the clinical records in each instance.

This report is designed to review the findings noted on the occlusal surfaces of the second permanent molar teeth of these children. This specific tooth area was chosen for study because of its ready availability for clinical examination,

*Presented at the 29th Annual Meeting of the International Association for Dental Research, French Lick Springs, Ind., March 17, 1951. (*J. D. Res.* **30**: 465, 1951).

This study was made possible by a grant from The Sugar Foundation.

Received for publication May 22, 1951, revised by authors Sept. 20, 1951.

the similarity of posteruptive tooth age of these teeth for the subject personnel used in the study, and the high susceptibility of such occlusal surfaces to caries initiation. Only those second molars were included which had been examined serially after eruption for at least 24 months, among children who had received semiannual recurrent dental examinations for four successive years. No child was included unless one or more of the second molars had been present in the mouth for at least three years of the study. The resultant group comprised 24 boys and 25 girls. The earliest dental examinations were made in July, 1946, and the most recent examinations used for consideration were made in August, 1950. The subjects were drawn from the population of a school for the feebleminded.[1, 2] These subjects had received minimal amounts of dental care prior to the beginning of the study, and little or none was offered during its course. Oral hygiene varied among the different subjects, but on the whole was decidedly poor.

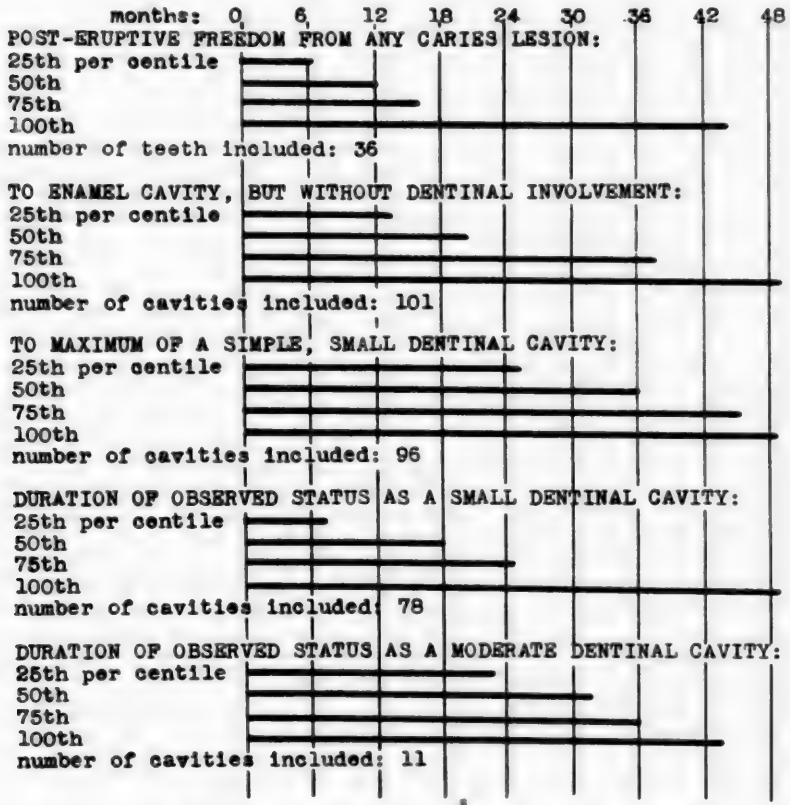

Fig. 1.—The lapse of time during which unfilled cavities on the occlusal surfaces of second permanent molar teeth retained the designated status relating to progression of dental caries. The values (expressed in months) represent the findings observed for the respective teeth holding the quartile positions within each subgroup.

PRESENTATION OF DATA

Our objective was to compare the extent of dental cavities in different second molar teeth, and in the same tooth at different periods of observation. To classify the degree of involvement, we have defined caries extent according to five successive categories of cavity size: (1) the tooth surface area being considered is free from any lesion which might properly be termed carious; (2) a minor enamel lesion is present, of such a nature that one may assume that it represents carious attack but where the dentin has not been attacked in so far as can be determined either clinically or from the radiograph; (3) a carious lesion is present which has involved both the enamel and dentin, but in which the lesion is small and confined; (4) a lesion similar in nature to the foregoing one is present, but its extent is greater; yet the architecture of the tooth is sufficiently well preserved that the tooth should be easily restorable through operative procedure; (5) extensive dentinal caries is present, endangering the tooth, with predictable loss of the tooth unless repaired promptly.

The status of each cavity was scored according to this classification to represent the findings at each dental examination, and then the time required for transition from one stage to others was determined. From the data so obtained, frequency distribution tables were constructed to indicate the variability in time required for various stages of transition.

Table I shows the number of dental cavities observed at the first and the last dental examinations. It also indicates the proportion of the total number of cavities which were found in the second molar teeth, and of that number the proportion which occurred on the occlusal surfaces. It will be noted that the occlusal surfaces of the second molar teeth accounted for about 20 per cent of all cavities in the mouths, and that about 80 per cent of all cavities in the second molar teeth occurred in the occlusal surfaces.

TABLE I
NUMBER OF DENTAL CAVITIES AT THE TIME OF THE FIRST AND LAST EXAMINATIONS

		NUMBER OF DENTAL CAVITIES			PER CENT OF TOTAL CAVITIES	
	NUMBER OF SUBJECTS	IN ALL TEETH	IN SECOND PERMANENT MOLARS	ON OCCLUSAL SURFACES OF SECOND MOLARS	IN SECOND PERMANENT MOLARS	ON OCCLUSAL SURFACES OF SECOND MOLARS
First examination	49	597	138	116	23	19
Last examination	49	1187	331	260	28	22

Fig. 1 indicates the length of time which elapsed while specific cavities retained the characteristics which placed them within their respective designated group, or else did not progress beyond a certain designated classification. The values are expressed in terms of months, as observed for the individual subjects who occupied the quartile positions. The data summarize the following observations. One or more carious lesions had been diagnosed on half of the occlusal surfaces within a year after the tooth's eruption and on three-fourths of the surfaces within 18 months. All these initial lesions were minor breaks in the continuity of the enamel, which presumably were carious. They arose pri-

marily from developmental pits and fissures. Once the initial lesion had been diagnosed as carious, long periods usually elapsed before the involvement had spread notably. With passage of time, 22 out of 82 such lesions became obviously noncarious, and it must be assumed that the diagnosis of caries in those areas was faulty at the start. The median length of time required for progression to a noteworthy enamel lesion was 20 months. To reach mild dentinal involvement took 3 years for the median cavity.

Certain cavities were well established when the children's mouths first were examined. Fig. 1 shows the lengths of time during which minor dentinal lesions remained in that status. The median time was 18 months. For cavities which already had reached moderate dentinal involvement when the child first was seen, the median duration of such status thereafter was 32 months. In many subjects, the cavities had not advanced beyond their initial status when the terminal examination was made; therefore the time periods listed can be considered minimal rather than truly representative.

In summary, our data indicate that without reparative or other dental therapy, cavities tended to remain almost static for periods of many months; that progression toward more severe caries involvement was not rapid in the great majority of cavities observed. The appearance of the initial enamel lesion, by contrast, was relatively prompt; three-fourths of the occlusal surfaces had developed lesions which were definitely or presumably carious in some degree within eighteen months after eruption of the tooth.

DISCUSSION

Because of the poor dental hygiene of our subjects and their scant dental reparative service, one might have predicted rapid progression of the individual caries lesion, rather than the slow advance which has been observed. Most of the children's mouths were habitually dirty. High salivary lactobacillus counts were the rule among the members of the group studied.[3] The fluoride content of the domestic water supply at the institution where the subjects were housed was low: numerous serial analyses made during the course of our study[1, 2] have failed to show more than 0.4 p.p.m. of fluoride. Moreover, the longer the children had been resident in the institution, the lower their caries scores had tended to be.[4] Mottling of enamel was not apparent. Thus, basing ones judgment on the customary explanations for the initiation of caries, one would have expected decay to advance rapidly under such adverse conditions.

On the other hand, what evidence do we have that untreated caries lesions in the mouth of the average child from any population group tend to progress any more rapidly in extent? Judgments cannot properly be drawn from the course of the child with rampant caries who is brought to the dental clinic specifically for treatment. To offset his rapid caries progression one can cite children whose initial status was similar, but whose untreated cavities have not progressed and consequently have not led the parents and the child back to the dentist for relief.

When circumstances permit, it is considered best dental practice to fill cavities once decay has become evident, before the process has had an opportunity

to destroy the tooth. The authors agree without reservation that such practice is sound from the viewpoint of therapy. But as a consequence of this practice, there are few data available relating to caries progression in unfilled cavities. To extend the available data it will be helpful if others who have the facilities at hand will record and publish their observations as to rates of caries progression in untreated cavities among different child populations.

In another publication, the authors have drawn attention to some common errors of estimation of rates of caries progression.[2] In making clinical studies of the effect of various agents as they may affect rates of caries advance, it is important to avoid as many of these errors as possible. One such error relates to the limitation of observation to the first appearance of new caries lesions. Our studies have shown that there is little relationship between the rate at which the earliest caries lesion becomes apparent and the rate of subsequent advance of the caries lesion. Moreover we have found that there is a high factor of error in the correct diagnosis of the early enamel defect. A series of equally skilled examiners may diagnose the same set of lesions differently: some as early caries, others as noncarious developmental defects. Conclusions based only on the appearance of new cavities are less defensible than are those which follow the serial course of the cavity as it evolves. Only the latter provides a true picture of the course of tooth decay.

For the purpose of clinical investigation, therefore, it is important to leave all test cavities unfilled and untreated throughout the whole period of observation. The placement of fillings in itself comprises a method of caries control, and interferes with the proper appraisal of any other agency being considered. Likewise it prevents estimation of the tendency toward caries progression in depth, as contrasted with estimation of the number of new areas of attack. Reliable judgment can be based only on the serial course of untreated individual caries lesions, with determination of the spontaneous variability of advance as contrasted with the degree of variability observed among those subjected to the effects of the agent being tested.

SUMMARY

This study deals with the length of time which elapsed before dental caries on the occlusal surface of second molar teeth progressed from one degree of severity to the next. The teeth studied received no operative or other therapeutic treatment during the course of the study. The value of the data to the clinician and to the dental investigator is discussed.

REFERENCES

1. Boyd, Julian D., Wessels, Kenneth E., and Cheyne, V. D.: Long-Term Studies of Dental Caries Progression Among Teen-Aged Inmates of a Custodial Institution, *J. Calif. D. A.* **26**: 30, 1950.
2. Boyd, J. D., and Wessels, K. E.: Epidemiologic Studies in Dental Caries, III: The Interpretation of Clinical Data Relating to Caries Advance, *Am. J. Pub. Health* **41**: 976, 1951.
3. Boyd, J. D., Wessels, K. E., and Cheyne, V. D.: Is the Lactobacillus Count a Valid Index of the Activity of Dental Caries? *Proc. Soc. Exper. Biol. & Med.* **71**: 533, 1949.
4. Boyd, J. D., and Cheyne, V. D.: Epidemiologic Studies in Dental Caries. II.: The Incidence of Caries Among Institutionalized Children, *J. Pediatrics* **31**: 306, 1947.

STUDIES ON THE EFFECT OF METALLIC SALTS ON ACID PRODUCTION IN SALIVA. I.

J. C. FORBES AND J. D. SMITH

Departments of Biochemistry and Chemistry, Medical College of Virginia, Richmond, Va.

IT IS generally believed that the production of acid by the action of microorganisms on fermentable carbohydrates in the mouth is a dominant factor in the development of dental caries. Consequently, a great deal of work has been done during the last few years in an effort to obtain a substance which would prevent or at least retard the rate of acid formation, and which could be used without danger of toxic reactions. Among the substances which have been recommended are urea,[1, 2] ammonium salts,[3] vitamin K,[4] quartenary ammonium salts,[5] and penicillin.[6]

At this time we wish to report on the marked inhibitory action of several metallic salts, especially those of copper and nickel, on the production of acid by saliva containing either glucose or sucrose. Saliva was collected by paraffin stimulation and sucrose added to give a 5 per cent concentration. Aliquots were then transferred to test tubes and various amounts of a solution of the salt being studied were added to all but the control tubes. All were then made up to the same volume by the addition of the necessary amount of water. The copper salts, which are insoluble in water, were dissolved in a small amount of 5 per cent monoethanolamine and then diluted with 9 volumes of water. The maximum amount of this solution added to 9 ml. of the saliva-sucrose solution was 1 ml., thus making a final maximum monoethanolamine concentration of only 0.05 per cent. The control tubes of these experiments contained an amount of monoethanolamine equal to the maximum present in the experimental tubes. All tubes were incubated at 37° C. and their pH values were determined with a Beckman pH meter at intervals thereafter. A number of determinations were run on each compound and the results obtained were in general agreement; however, in order to conserve space, the results of only one experiment per compound are reported. Glucose was substituted for sucrose in a few experiments and comparable results were obtained. A number of other copper salts besides those reported in Table I have been been studied and all showed the same general inhibition when compared at the same copper level. These included copper butyrate, caprylate, oleate, stearate, 9-hendecynoate, 2-dodecynoate, salicylate, benzoate, mandelate, cinnamate, and ethyl acetoacetate.

It will be seen from the results that a copper concentration of 3 to 4 mg. per 100 ml. of saliva was sufficient to completely prevent acid formation, while a concentration of 0.25 mg. per 100 ml. gave fairly marked inhibition. Un-

Received for publication, June 13, 1951.

TABLE I

EFFECT OF COPPER SALTS UPON ACID PRODUCTION IN SALIVA

TIME OF INCUBATION (HRS.)	CONCENTRATION OF COPPER MG./100 ML. 4 (pH)	3 (pH)	2 (pH)	1 (pH)	0.5 (pH)	0.25 (pH)	0 (pH)	SUBSTANCE USED
22	7.9	7.5	7.6	6.3	5.2	4.7	4.3	Copper acetate
48	7.7	7.6	7.5	4.5	4.4	4.4	3.9	
24	–	7.6	7.3	6.3	5.7	5.0	4.3	Copper propionate
52	–	7.7	6.0	4.5	4.4	4.3	3.8	
24	–	8.4	8.2	6.9	6.2	4.9	4.5	Copper laurate*
48	–	8.4	8.1	5.3	4.9	4.6	4.0	
24	7.3	7.4	7.3	6.2	5.3	4.9.	4.4	Copper salicylate
48	7.8	7.5	6.8	5.1	4.6	4.4	4.1	
22	7.9	7.7	7.6	6.3	5.2	4.8	4.3	Copper sulfate
48	7.8	7.8	5.4	4.4	4.2	4.2	3.8	
24	7.5	7.3	7.3	6.2	5.1	4.9	4.4	Copper chloride
48	7.7	7.6	7.0	4.7	4.4	4.3	4.1	
24	8.1	7.9	6.9	5.6	5.2	4.7	4.2	Copper saccharinate
48	8.1	7.9	6.1	4.5	4.5	4.3	3.8	

*This was dissolved in a weak solu*ion of monoethanolamine.

recorded experiments with several copper salts showed that concentrations as low as 0.1 mg. of copper per 100 ml. exerted demonstrable inhibitory action, the effect being especially evident during the first 24 hours of incubation.

Nickel salts have also been studied rather extensively and found to be in general almost as active as copper salts. A few of these results along with some obtained on other metals are shown in Table II. It will be seen that silver, gold, and mercury salts are quite active, comparing favorably to copper and nickel. Magnesium, cobalt, manganese, aluminum, iron, and chromic salts were found to have little or no activity. Platinum and palladium showed moderate activity, being superior to zinc but below that of copper.

The results indicate that the inhibitory effect on acid production is primarily a function of the metal content. It is, however, possible that some metal derivative may be found in which this is not the case.

TABLE II

EFFECT OF VARIOUS METALLIC SALTS UPON ACID PRODUCTION IN SALIVA

TIME OF INCUBATION (HRS.)	CONCENTRATION OF METAL MG./100 ML. 6 (pH)	4 (pH)	2 (pH)	1 (pH)	0 (pH)	SUBSTANCE USED
23	7.0	6.9	6.7	6.8	4.1	Nickel propionate
51	7.1	6.7	5.5	4.9	3.7	
24	7.2	7.4	7.5	7.2	4.2	Nickel chloride
48	8.0	8.1	8.0	6.1	4.0	
24	7.3	7.4	7.4	6.7	4.4	Nickel iodide
50	7.6	7.5	7.3	5.3	4.0	
24	7.6	7.6	7.6	7.5	4.2	Silver nitrate
48	8.1	8.1	8.1	8.1	4.0	
22	7.6	7.4	7.4	6.8	4.5	Gold chloride
43	7.3	6.5	6.5	6.0	4.1	
22	7.8	7.8	7.4	7.2	4.5	Mercuric chloride
43	7.9	8.0	7.4	6.8	4.1	
24	5.2	5.0	4.5	4.2	4.2	Zinc sulfate
48	4.7	4.5	4.2	4.0	3.9	
22	4.5	4.5	4.5	4.5	4.5	Magnesium chloride
43	4.2	4.2	4.2	4.2	4.1	

SUMMARY

The effect of various metallic salts upon acid production in saliva containing sucrose has been studied. Copper, nickel, gold, silver, and mercury salts exerted the greatest inhibitory action on acid production. Magnesium, cobalt, manganese, aluminum, iron, and chromic salts showed little or no activity. Copper at a concentration of 0.25 mg. per 100 ml. of saliva containing sucrose definitely inhibited acid formation while a concentration of 3 to 4 mg. per 100 ml. of saliva caused complete inhibition of acid production.

REFERENCES

1. Stephan, R. M.: *J. D. Res.* **22**: 63, 1943.
2. Wach, E. C., O'Donnell, J. F., and Hine, M. K.: *J. A. D. A.* **29**: 61, 1942.
3. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, E. C.: Am. J. Orthodontics **33**: 80, 1947.
4. Burrill, D. Y., Calandra, J. C., Tilden, E. B., and Fosdick, L. S.: *J. D. Res.* **24**: 273, 1945.
5. Stephan, R. M., and Miller, B. F.: *J. D. Res.* **22**: 53, 1943.
6. Zander, H. A.: *J. A. D. A.* **40**: 569, 1950.

STUDIES ON THE EFFECT OF METALLIC SALTS ON ACID PRODUCTION IN SALIVA. II.

J. C. FORBES AND J. D. SMITH

Departments of Biochemistry and Chemistry, Medical College of Virginia, Richmond, Va.

IN THE first paper of this series we have reported the marked inhibitory action of various metallic salts, including those of copper and nickel, on the formation of acid in saliva containing either glucose or sucrose.[1] The present study deals with (a) the effect of washing the mouth and brushing the teeth with an aqueous solution of these agents on the production of acid by subsequent saliva collections after addition of sucrose and incubation at 37° C., and (b) the apparent bactericidal action of copper and nickel salts on the acidogenic organisms in saliva.

EXPERIMENTAL

Effect of Mouth Washing and Teeth Brushing.—These results are divided into two sections: (a) The effect of a single brushing and washing with the inhibitor solution; (b) the effect of repeated brushings and washings. In the first case saliva was collected with paraffin stimulation in the morning after reaching the laboratory, then the mouth was washed with some brushing of the teeth for approximately 5 minutes using three separate portions of the inhibitor solution. After an additional 3 minutes, the mouth was washed with water several times so as to remove the excess inhibitor. Saliva was collected at intervals thereafter in the usual way during a 3-hour period. Sucrose was added to all the tubes so as to give approximately a 5 per cent concentration and the tubes were incubated at 37° C. for 24 hours. Several studies were run with each inhibitor and the results obtained were always in good general agreement. The averages of the various experiments in each series are shown in Figs. 1 and 2. It will be seen from Fig. 1 that a single washing and brushing with a copper solution of only 0.0035 M (22 mg. per 100 milliliters) exerted an effect for at least 3 hours. A 0.01 M (63 mg. per 100 milliliters) solution as shown in Fig. 2 is still more effective. Copper chloride was found to have a greater activity than nickel chloride. We have found this to be the general tendency among the various copper and nickel salts studied, although the differences were not always pronounced. Studies with copper concentrations of 0.0015 M (9.5 mg. per 100 milliliters) showed good inhibition of acid production but the effect did not usually last as long as 3 hours. However, the duration of effect is considerably prolonged if the first saliva collection is postponed for several hours. This may be due to the mechanical removal during the saliva collection of inhibitor which had been adsorbed or chemically combined with organic matter in the mouth.

Received for publication, June 13, 1951.

Effect of Repeated Washings and Brushings.—The purpose of this experiment was to determine if mouth washing as already described would reduce the concentration of the acidogenic organisms of the saliva over a 24 hour period, as measured by the amount of acid produced when the saliva was incubated with sucrose. Saliva collections were made at approximately 7:30 A.M., 12 noon, 6 P.M., and 11 P.M. Breakfast was around 7:45, lunch at 12:30, and dinner at 6:30. The mouth was washed shortly after each meal and at 11:10 P.M., just before retiring. During the control period at the beginning of the experiment, the teeth were brushed and mouth washed with water instead of with the inhibitor solution. The samples collected at 6 P.M., 11 P.M., and 7:30 A.M. were kept until the 12 noon collection when sucrose was added to all and the tubes incubated together for 23 hours. Tests have shown that the pH reached by untreated saliva after addition of either glucose or sucrose was not measurably affected by the saliva standing at room temperature for 24 hours prior to addition of the sugar and incubation. It will be seen from the results which are recorded in Fig. 3 that the teeth brushing and mouth washing either caused a marked reduction in the number of acidogenic organisms in the mouth or reduced in some other way their acid-producing potential.

Bactericidal Studies.—A large amount of saliva was collected in the usual manner, thoroughly mixed and 10 ml. portions were transferred to sterilized 50 ml. centrifuge tubes, care being taken not to get the saliva on the side of the tubes. Aliquots of a standard solution of the inhibitor were added to give the desired inhibitor concentration and the contents were mixed by gentle tapping. After various periods of time, 0.5 ml. of the treated saliva was transferred with sterile precautions to a culture tube. Control tubes were similarly prepared using 0.5 ml. of the same saliva without inhibitor. The tubes were then incubated at 37° C. for the specified period of time after which their pH was measured with a Beckman pH meter. The culture tubes were prepared as follows: Saliva was collected with paraffin stimulation and sucrose added to make a 5 per cent solution. Aliquots of 9.5 ml. were transferred to test tubes, plugged with cotton and sterilized at 15 pounds pressure for 15

TABLE 1

BACTERICIDAL ACTION OF COPPER AND NICKEL CHLORIDE ON THE ACIDOGENIC ORGANISMS IN SALIVA

DURATION OF INCUBATION (HOURS)	CONCENTRATION OF INHIBITOR TIMES 10^{-3} M				TIME SALIVA IN CONTACT WITH INHIBITOR
	2.0 (pH)	1.5 (pH)	1.0 (pH)	0 (pH)	
48	--	6.8	4.6	4.5	$NiCl_2$ for 15 minutes
48	--	7.9	4.7	4.4	As above for 1 hour
48	--	8.0	6.5	4.3	As above for 23 hours
48	5.5	5.1	4.7	4.4	As above for 15 minutes
48	5.9	5.1	4.7	4.4	As above for 1 hour
48	8.0	7.6	5.2	4.4	As above for 24 hours
48	--	4.9	4.9	4.5	$CuCl_2$ for 15 minutes
48	--	4.8	4.9	4.4	As above for 1 hour
48	--	7.9	8.0	4.3	As above for 23 hours
48	6.9	5.1	4.9	4.4	As above for 15 minutes
48	8.0	4.9	5.0	4.4	As above for 1 hour
48	8.0	5.3	6.9	4.4	As above for 24 hours

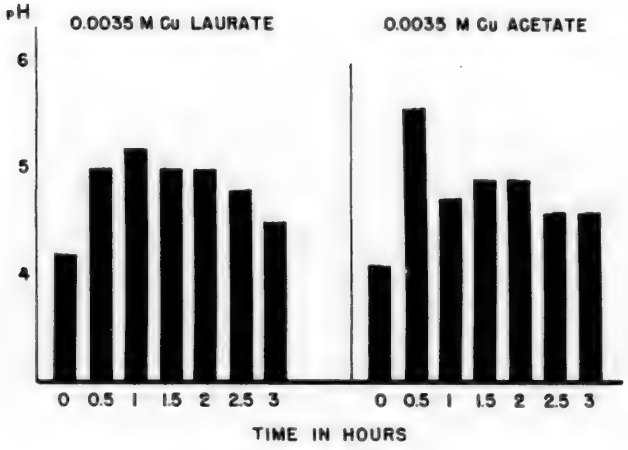
pH
0.0035 M Cu LAURATE
0.0035 M Cu ACETATE
6
5
4
0 0.5 1 1.5 2 2.5 3
0 0.5 1 1.5 2 2.5 3
TIME IN HOURS

Fig. 1

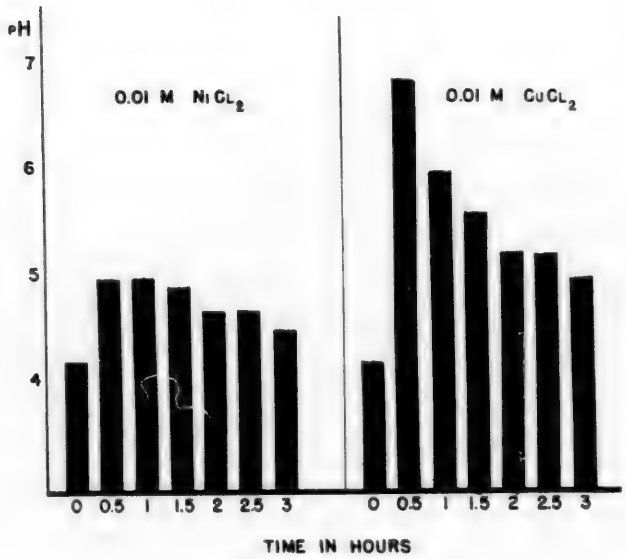
pH
7
6
5
4
0.01 M $NiCl_2$
0.01 M $CuCl_2$
0 0.5 1 1.5 2 2.5 3
0 0.5 1 1.5 2 2.5 3
TIME IN HOURS

Fig. 2.

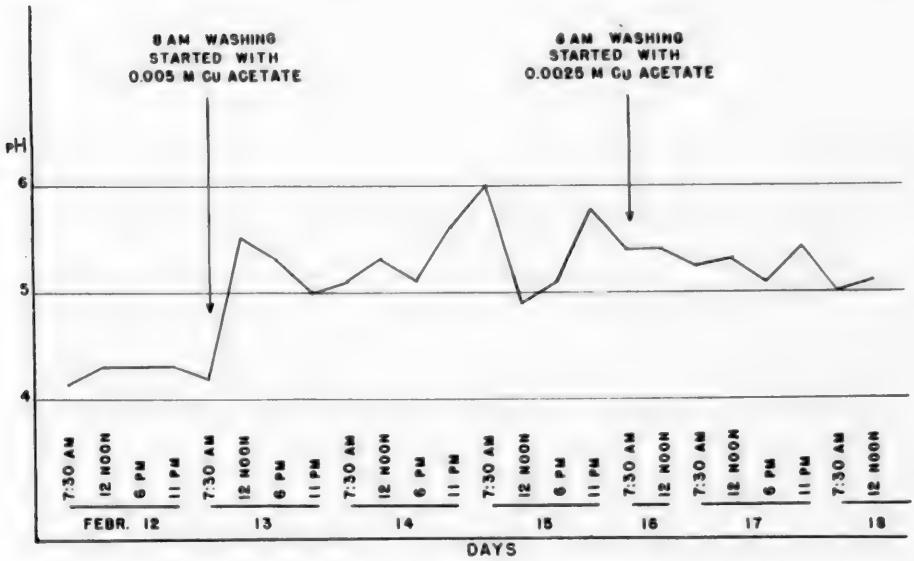

Fig. 3.

minutes. A few of the experimental results are shown in Tables I and II. It will be seen from Table I that a concentration of 0.002 M of nickel or copper chloride either destroyed the acidogenic organisms or completely inhibited in some other way their acid-producing power when exposed to it for about 24 hours. Even an exposure of 15 minutes resulted in the apparent destruction of many of the organisms. It should be noted that the maximum concentration of inhibitor in any of the culture tubes was only 0.0001 M (0.63 mg. per 100 milliliters in the case of copper). As reported in Part I of this study, this concentration although exerting a distinct inhibitory effect on acid formation is well below that necessary for complete inhibition.[1] Results obtained with copper laurate dissolved in triethanolamine are shown in Table II. The results

TABLE II

BACTERICIDAL ACTION OF COPPER LAURATE-TRIETHANOLAMINE SOLUTION OF THE ACIDOGENIC BACTERIA IN SALIVA

DURATION OF INCUBATION (HOURS)	CONCENTRATION OF INHIBITOR TIMES 10^{-3} M					TIME SALIVA IN CONTACT WITH INHIBITOR
	1.72 (pH)	.86 (pH)	.43 (pH)	.17 (pH)	0 (pH)	
47	8.0	5.6	5.3	4.6	4.1	70 minutes
48	7.8	5.9	5.0	4.9	4.3	27 hours
47	7.7	4.7	4.5	4.5	4.1	70 minutes
48	8.0	5.2	4.9	4.8	4.3	27 hours
48	7.6	5.6	4.8	4.8	4.1	1 hour
72	7.9	7.9	7.6	4.7	4.2	24 hours
96	5.4	5.0	4.5	4.0	4.0	1 hour
96	8.3	8.1	4.9	4.0	3.8	48 hours

on the whole are roughly comparable to those obtained with copper chloride. The maximum amount of triethanolamine present in the culture tubes was only 0.02 per cent, consequently neutralization of acid by this base cannot be responsible for the absence of a drop in the pH of the tubes which had been inoculated with saliva exposed to the higher concentration of inhibitor. The minimum concentration of inhibitor necessary for apparent destruction of the organisms during a 24 hour exposure varied slightly in different experiments. This difference may be due to variations in the number of organisms and the amount of proteinaceous material in the saliva.

All of the copper compounds, which have been tested, have a metallic taste and their continued use four times a day as a mouthwash in the higher concentrations cause a dark deposit along the longitudinal fissure of the tongue. This deposit, however, disappears in a few days after the mouth washing is discontinued. The saccharinate has the most pleasant taste of all the copper salts thus far tested and more work is being carried out with this preparation.

SUMMARY

Data have been presented showing that when the mouth is washed and the teeth brushed with an aqueous solution of a copper or nickel salt, there is marked reduction in the amount of acid produced in saliva samples collected over the next few hours. This effect can be maintained over a 24-hour period if the brushing and washing are repeated after each meal and again shortly before retiring. Copper or nickel concentrations of 0.002 M apparently destroy the acidogenic organisms of saliva if they are exposed to it for as long as 24 hours.

REFERENCE

1. Forbes, J. C., and Smith, J. D.: *J. D. Res.* **31**: 129, 1951. (This issue.)

THE COPPER AND COBALT LEVELS OF HUMAN SALIVA AND DENTAL CARIES ACTIVITY

SAMUEL DREIZEN, D.D.S., HENRY A. SPIES, JR., B.S., AND TOM D. SPIES, M.D.

Northwestern University Studies in Nutrition at the Hillman Hospital, Birmingham, Ala., Department of Nutrition and Metabolism

IT IS well known that many mineral elements are present in human tissues and tissue fluids in widely varying amounts. Those which occur in quantities equal to or less than that of iron have been arbitrarily designated "trace elements."[1] Six of these, namely, iron, iodine, copper, cobalt, manganese, and zinc, have been shown to be essential to animal life.[2] Investigations conducted on the bacterial level using chemically defined synthetic media indicate that not all species of organisms require the same trace minerals, and that in some instances those which are beneficial to the host may be detrimental to bacteria.

As producers of acids capable of decalcifying tooth structure, certain bacteria have been long identified with the pathogenesis of human dental caries. Extensive studies have been made in an effort to elucidate the factors restricting the growth of such organisms in human saliva. That some of the trace elements might affect dental caries activity is suggested by the findings of MacLeod and Snell[3] and of Stephan.[4] The former investigators showed that potassium, manganese, and, for some species, magnesium are essential for the optimal growth of lactic acid-producing bacteria in a medium containing all of the other known growth essentials of these organisms. Stephan[4] found that 0.01 M concentrations of Cu^{++}, Co^{++}, Ni^{++}, Mo^{++}, and F^{-} prevented the growth of a large number of strains of oral acidogenic lactobacilli, streptococci, and staphylococci when added to a synthetic medium seeded with these organisms. Since each of these five trace elements influences enzyme systems involved in carbohydrate degradation,[5] it seemed possible that their presence in saliva might affect dental caries activity. In order to test this hypothesis, human saliva has been analyzed for Cu^{++}, Co^{++}, Ni^{++}, and Mo^{++},* and a study has been made of the degree to which those present affect acid production in saliva and the growth of an oral strain of *Lactobacillus acidophilus*.

MATERIALS AND METHODS

Fourteen members of the clinic staff contributed saliva samples for the qualitative and quantitative analyses for the trace elements. The former consisted of spectrographic examinations of 21 samples of paraffin-stimulated saliva obtained from 11 of the 14 subjects, each of whom had clinical and laboratory evidence of dental caries susceptibility. In these determinations, 50 c.c. of whole saliva from each sample were evaporated to dryness, ashed at 500° C. to

These studies were supported by grants from the Clayton Foundation for Research.
Received for publication, July 23, 1951.

*In view of the recently reported findings of Martin and Hill[6] that the fluorine content of saliva is not related to oral lactobacillus counts and to the prevalence of dental caries, an analysis of the fluorine content of saliva was not included in this investigation.

1,000° C., and tested for Cu^{++}, Co^{++}, Ni^{++}, and Mo^{++}. Ten of the 14 subjects, 7 of whom were caries active and 3 of whom were caries inactive, provided 85 samples of paraffin-stimulated saliva for the quantitative analyses. Forty-eight of the samples were analyzed for Cu^{++}, and 37 of the samples were analyzed for Co^{++}. Each sample was collected in a sterile Pyrex flask within two hours after breakfast. Eight of the 10 subjects contributed saliva for the copper determinations, and 7 of the 10 for the cobalt determinations. At least three specimens of saliva were submitted by each participant for each analysis of copper and cobalt. The copper content of whole saliva was determined by the colorimetric method of van Ravesteyn.[7] Cobalt was determined by the colorimetric method of MacPherson and Stewart.[8] Since in the latter method, copper and iron may also react with the nitroso-R-salt to form colored complexes which interfere with the estimation of cobalt copper, and iron were first removed by the method suggested by MacPherson and Stewart.[8] Quantitative tests for copper and cobalt were made on two 6 c.c. aliquots from each saliva sample. The cobalt and copper content of each sample was calculated from standard curves developed by the use of known amounts of soluble salts of each of these elements in redistilled water.

The effect of the removal of copper and cobalt from saliva on dental caries activity was determined by the addition of 0.5 c.c. of a 0.01 M solution of sodium diethyldithiocarbamate. This compound removes both cobalt and copper from active participation in a biological system by forming insoluble and non-diffusible salts with these elements.[9] Sodium diethyldithiocarbamate was added to 10 c.c. aliquots from ten different samples of stimulated whole saliva. The aliquots treated with the chelating agent were subjected to the Fosdick, Hansen, and Epple[10] test for dental caries activity, and the results were compared to those obtained for untreated aliquots from the same samples of saliva.

To determine the effect of added copper and cobalt on acid production in human saliva, soluble salts of these elements containing 20.0 μg of Cu^{++} and 19.7 μg of Co^{++} were added to 10 c.c. aliquots from 12 samples of stimulated whole saliva obtained from patients with active dental caries. These values corresponded to approximately 4 times the maximum amount of copper, and 20 times the maximum amount of cobalt found in saliva.

The effect of copper and cobalt in amounts equal to the maximum concentration present in saliva on the growth of an oral strain of *L. acidophilus* was determined by preparing pour plates of Kulp's tomato juice agar each of which contained a central well. The agar was inoculated with one loopful of a 24-hour yeast extract-dextrose broth culture of *L. acidophilus*. The test organism was recovered from the saliva of a patient with active and rampant dental caries. Soluble salts of copper and cobalt in amounts equal to the maximum concentrations present in saliva were added to the central wells either singly or in combination. The plates were incubated at 37° C. for 72 hours after which they were examined for zones of inhibition.

The ability of copper and cobalt to replace trace elements known to be essential for the growth of the oral *L. acidophilus* was determined by substituting these elements for potassium, manganese, and magnesium in the chemi-

cally defined synthetic medium of Landy and Dicken.[11] This medium contains all of the known growth requirements of this organism. The tubes were inoculated with an oral strain of *L. acidophilus* and incubated at 37° C. Acid production was measured at the end of 24, 48, and 72 hours by titration with 0.1 N NaOH.

OBSERVATIONS

The Presence of Copper, Cobalt, Nickel and Molybdenum in Human Whole Saliva.—The results of the spectrographic analyses for copper, cobalt, nickel, and molybdenum are presented in Table I. None of the 21 samples tested contained detectable amounts of nickel and molybdenum. Each contained copper and 3 contained traces of cobalt, the latter occurring in both samples submitted by J. H. and in one of the two samples obtained from F. S.

TABLE I
DISTRIBUTION OF TRACE ELEMENTS ANTAGONISTIC TO ORAL ACIDOGENIC BACTERIA IN 21 SAMPLES OF HUMAN WHOLE SALIVA AS DETERMINED BY SPECTROGRAPHIC ANALYSIS

SUBJECT	TRACE ELEMENT			
	COPPER	COBALT	NICKEL	MOLYBDENUM
H. S.	+	–	–	–
A. R.	+	–	–	–
F. S.	+	–	–	–
S. H.	+	–	–	–
B. N.	+	–	–	–
N. D.	+	–	–	–
J. H.	+	–	–	–
E. L.	+	–	–	–
A. S.	+	–	–	–
S. D.	+	–	–	–
A. S.	+	–	–	–
A. R.	+	–	–	–
F. S.	+	+	–	–
D. S.	+	–	–	–
J. H.	+	+	–	–
N. D.	+	–	–	–
A. S.	+	–	–	–
D. S.	+	–	–	–
F. S.	+	–	–	–
S. D.	+	–	–	–
J. H.	+	+	–	–

Copper and Cobalt Levels in Human Whole Saliva.—The copper and cobalt levels of the 48 and 37 samples of whole saliva which were analyzed quantitatively for copper and cobalt, respectively, are shown in Table II. The copper concentrations ranged from 10.00 to 47.50 μg. per 100 c.c. of whole saliva. Cobalt concentrations ranged from 0.00 to 12.53 μg. per 100 c.c. of whole saliva. The mean saliva copper concentrations of the 8 subjects tested ranged from 23.25 to 28.00 μg. per 100 c.c. The mean saliva cobalt concentrations of the 7 subjects studied ranged from 0.60 to 4.18 μg. per 100 c.c. Each of the 48 samples tested for copper contained this element in amounts detectable by the method used. The mean copper concentration of the 48 samples was 25.60 μg. per 100 c.c. of whole saliva. Of the 37 samples of saliva analyzed for cobalt, 27 (73 per cent) did not contain cobalt in amounts detectable by the method used. The mean concentration of cobalt in the 10 samples in which this element was

TABLE II
COPPER AND COBALT LEVELS OF HUMAN WHOLE SALIVA

SUBJECT	SEX	CARIES ACTIVITY*	COPPER			COBALT		
			NO. OF SAMPLES	RANGE (μG./100 C.C.)	MEAN (μG./100 C.C.)	NO. OF SAMPLES	RANGE (μG./100 C.C.)	MEAN (μG./100 C.C.)
1. A. S.	M	+++	18	10.00-47.50	25.25	7	0.00-12.53	3.10
2. A. R.	F	++	8	15.00-40.00	25.25	4	0.00- 4.18	1.06
3. T. B.	F	++	6	17.50-42.50	25.25			
4. S. D.	M	-	4	22.50-37.50	28.00	7	0.00- 4.20	0.60
5. C. B.	M	++	3	20.00-25.00	23.25			
6. B. N.	M	+++	3	15.00-37.50	25.00	3	0.00- 7.52	2.51
7. M. K.	F	-	3	22.50-30.00	25.75			
8. J. H.	M	-	3	17.50-37.50	26.75	8	0.00- 4.51	1.13
9. H. B.	M	++++				5	0.00-11.36	4.18
10. W. M.	M	++++				3	0.00- 7.52	2.51

*Fosdick, Hansen, and Epple Classification.

present was 7.04 μg. per 100 c.c. At least one sample from each subject contained a trace of cobalt. The variations in the copper and cobalt levels of the saliva samples collected from each subject on different days was substantially greater than the variations in the mean saliva concentrations of these elements in the different subjects. No correlation was observed between the copper and cobalt levels and the dental caries activity of the samples analyzed.

Effect of the Removal of Cu^{++} and Co^{++} on Acid Production in Human Saliva.—The effect of the removal of copper and cobalt on acid production in saliva by the addition of the chelating agent, sodium diethyldithiocarbamate, is shown in Table III. In the 10 instances in which this compound was added to samples of saliva with demonstrable caries activity, acid production was increased in 4 and decreased in 6. In all but two cases, B. E. and A. R., however, the differences between the treated and untreated aliquots could not be interpreted as significant.

TABLE III
EFFECT OF REMOVAL OF Cu^{++} AND Co^{++} ON ACID PRODUCTION IN HUMAN SALIVA

SUBJECT	CARIES ACTIVITY (UNTREATED SALIVA)	GAIN IN SOLUBLE CA (MG./10 C.C. SALIVA)	CARIES ACTIVITY (TREATED SALIVA*)	GAIN IN SOLUBLE CA (MG./10 C.C. SALIVA)
1. T. J. F.	+	0.38	-	0.06
2. S. E.	+++	1.25	++	0.81
3. S. S.	+++	1.08	+++	1.26
4. B. E.	++++	3.26	+++	1.33
5. S. D.	±	0.15	+	0.28
6. A. R.	++++	3.35	+++	1.06
7. M. M.	+++	1.48	+++	1.75
8. A. S.	+++	1.75	+++	1.70
9. E. B.	+++	1.10	+++	1.60
10. R. G.	++	0.51	++	0.80

*Treated by addition of 0.5 c.c. 0.01M sodium diethyldithiocarbamate.

Effect of the Addition of Cu^{++} and Co^{++} on Acid Production in Human Saliva.—Table IV shows the effect of the addition of Cu^{++} and Co^{++} in amounts equal to approximately 4 and 20 times, respectively, the maximum concentration present in saliva on acid production in this fluid. Acid production was com-

pletely inhibited in 2 and partially inhibited in 4 of the 6 samples to which Cu^{++} was added. Similar results were obtained for the 6 samples to which Co^{++} was added. In all instances the decrease in acid production was substantial.

TABLE IV
EFFECT OF THE ADDITION OF Cu^{++} AND Co^{++} ON ACID PRODUCTION IN HUMAN SALIVA*

PATIENT	AMOUNT ADDED/ 10 C.C. SALIVA	CARIES ACTIVITY (UNTREATED SALIVA)	GAIN IN SOLUBLE CA (MG./10 C.C. SALIVA)	CARIES ACTIVITY (TREATED SALIVA)	GAIN IN SOLUBLE CA (MG./10 C.C. SALIVA)
1. S. B.	20 μg Cu^{++}	+++	2.20	+++	1.35
2. C. B.	20 μg Cu^{++}	+++	1.50	+++	1.20
3. R. A.	20 μg Cu^{++}	+	0.39	-	0.09
4. W. M.	20 μg Cu^{++}	+++	1.28	±	0.14
5. M. H.	20 μg Cu^{++}	+++	1.63	++	0.86
6. E. B.	20 μg Cu^{++}	++	0.71	-	0.00
7 H. B.	19.7 μg Co^{++}	++++	3.20	++++	2.15
8. M. He.	19.7 μg Co^{++}	++++	2.95	++++	1.11
9. A. S.	19.7 μg Co^{++}	++++	2.06	++	0.97
10. J. S.	19.7 μg Co^{++}	++	0.59	-	0.00
11. W. K.	19.7 μg Co^{++}	++++	2.26	-	0.01
12. W. L.	19.7 μg Co^{++}	++++	3.46	++++	2.25

*Added as $CuSo_4$ and $CoCl_2$.

Cu^{++} and Co^{++} as Replacements for Essential Trace Elements in the Nutrition of an Oral Strain of Lactobacillus Acidophilus.—The inability of cobalt and copper to influence the growth and acid production of an oral strain of *L. acidophilus* is shown in Table V. The addition of these ions either singly or in combination in amounts corresponding to those present in saliva failed completely to inhibit the growth of this organism in Kulp's tomato juice agar. The presence of Co^{++} or Cu^{++} as replacements for Mg^{++}, Mn^{++}, or K^{+} in the test medium failed either to stimulate or to inhibit the growth of the *L. acidophilus* in a medium containing all of the other known growth factors of this organism.

DISCUSSION

Of the 4 trace elements, copper, cobalt, nickel, and molybdenum, previously shown to be inhibitory to oral acid-producing bacteria when added in vitro in fairly large concentrations, only copper and cobalt were detected in human

TABLE V
COPPER AND COBALT AS REPLACEMENTS FOR ESSENTIAL TRACE ELEMENTS IN THE NUTRITION OF AN ORAL STRAIN OF LACTOBACILLUS ACIDOPHILUS

TUBE	ACID PRODUCTION (C.C. 0.1 N NaOH)		
	24 HOURS	48 HOURS	72 HOURS
1. Unmodified L.D.*	4.44	12.15	13.57
2. L.D.—Mg^{++}	2.52	6.88	8.72
3. L.D.—Mg^{++} + Cu^{++}	2.00	5.57	7.70
4. L.D.—Mg^{++} + Co^{++}	2.36	5.91	8.77
5. L.D.—Mn^{++}	2.80	6.00	8.60
6. L.D.—Mn^{++} + Cu^{++}	2.42	5.74	8.07
7. L.D.—Mn^{++} + Co^{++}	2.34	6.31	8.81
8. L.D.—K^{+}	0.00	0.00	0.00
9. L.D.—K^{+} + Cu^{++}	0.00	0.00	0.00
10. L.D.—K^{+} + Co^{++}	0.00	0.00	0.00

*L.D. = Landy and Dicken medium.

whole saliva by the methods used. The concentration of copper greatly exceeded that of cobalt, and was approximately 25 to 30 per cent as great as that in blood. The presence of copper in all samples of saliva examined for this element suggests that copper is a normal constituent of human whole saliva. The absence of cobalt from 27 of the 37 samples tested for this element indicates that cobalt is not a regular constituent of human saliva.

The copper and cobalt levels of saliva seem to bear no relationship to dental caries activity. The removal of these elements by chelation did not materially affect acid production in saliva. When added to a synthetic medium in the amounts present in saliva, these elements failed to influence the growth of an oral strain of *L. acidophilus*. The concentrations of Co^{++} and Cu^{++} required to inhibit acid production in human whole saliva greatly exceeds those found to be actually present in this medium.

SUMMARY AND CONCLUSIONS

Of the 4 trace elements antagonistic to the growth of oral acidogenic bacteria in synthetic media, saliva contains copper regularly, cobalt occasionally, and nickel and molybdenum not at all. Copper was present in amounts ranging from 10.00 to 47.50 mg. per 100 cubic centimeters in each of the 48 samples of saliva analyzed for this element. Cobalt was present in 10 of 37 samples, the maximum concentration being 12.53 mg. per 100 cubic centimeters of saliva. In vitro studies clearly demonstrate that in the maximum quantities available in saliva, copper and cobalt do not influence dental caries activity as measured by acid production in saliva and the growth of an oral strain of *L. acidophilus*.

REFERENCES

1. McCollum, E. V., Orent-Keiles, E., and Day, H. G.: The Newer Knowledge of Nutrition, New York, 1939, Macmillan Company.
2. Shils, M. E., and McCollum, E. V.: The Trace Elements in Nutrition, *J. A. M. A.* **120**: 609, 1940.
3. MacLeod, R. A., and Snell, E. E.: Some Mineral Requirements of Lactic Acid Bacteria, *J. Biol. Chem.* **170**: 351, 1947.
4. Stephan, R. J.: In Vitro Studies of the Effects of Some Chemical Substances on the Growth of Oral Microorganisms and on Their Ability to Dissolve Tooth Salts, *J. D. Res.* **28**: 652, 1949.
5. Sumner, J. B., and Somers, G. F.: Chemistry and Methods of Enzymes, New York, 1947, Academic Press, Inc.
6. Martin, D. J., and Hill, I. N.: The Evanston Dental Caries Study. V. The Fluorine Content of Saliva and Its Relationship to (A) Oral Lactobacillus Counts and (B) The Prevalence of Dental Caries, *J. D. Res.* **29**: 291, 1950.
7. van Ravesteyn, A. H.: Metabolism of Copper in Man, *Acta Med. Scandinav.* **118**: 163, 1944.
8. MacPherson, H. T., and Stewart, J.: The Photometric Estimation of Cobalt, *J. Biochem.* **32**: 763, 1938.
9. Albert, A., and Gledhill, W. S.: The Choice of a Chelating Agent for Inactivating Trace Metals, *J. Biochem.* **41**: 529, 1947.
10. Fosdick, L. S., Hansen, H. S., and Epple, C.: Enamel Decalcification by Mouth Organisms Suggested Test for Caries Susceptibility, *J. A. D. A.* **24**: 1275, 1937.
11. Landy, M., and Dicken, D. M.: A Microbiological Assay Method for Six B Vitamins Using Lactobacillus Casei and Medium of Essentially Known Composition, *J. Lab. & Clin. Med.* **27**: 1082, 1942,

FRACTURING OF THE MOLAR TEETH IN CARIES-SUSCEPTIBLE AND CARIES-RESISTANT ALBINO RATS (RATTUS NORVEGICUS)

E. C. NAKFOOR,* H. R. HUNT, AND C. A. HOPPERT

From the Departments of Zoology and Chemistry, Michigan State College, East Lansing, Mich.

THE researches of Hunt and Hoppert and their graduate students have sought to discover some of the factors that influence the development of caries in the lower molar teeth of albino rats. A caries-susceptible strain and a caries-resistant strain have been created since 1937 by means of phenotypic selection, progeny testing of breeding pairs, and brother by sister inbreeding. The caries-producing diet of Hoppert, Webber, and Canniff[1, 2] (66 per cent coarsely ground hulled rice, 30 per cent whole milk powder, 3 per cent alfalfa leaf meal, and 1 per cent sodium chloride) has provided a constant diet throughout the experiments. About 8,000 rats have been observed to date. The proof is conclusive that hereditary differences are important in the causation of caries in the lower molars of rats.[3-8] Use of a tooth facilitates the carious process,[8] sex is not an important causative factor,[9] and age appears to increase resistance more or less.[10] The appearance of rat caries is delayed by grinding the rice component of the ration finer than in the standard ration generally used.[10] The secretion of the parotid gland does not appear to be of great importance in promoting or delaying the formation of caries (data unpublished).

Robinson[11] has suggested that the caries in these two strains of rats may have been initiated by fracturing of the teeth. The coarse, hard rice particles might be the cause of such injury. Rosebury[12, 13] has suggested that true caries in rats can be identified as a rule only by microscopic examination of tooth sections, and is to be distinguished from other lesions in the teeth, some of which are initiated by fracturing.

The objective of the investigation reported in this paper was to compare the resistance to experimentally produced fracturing of the lower molar teeth of the caries-susceptible inbred rats with resistance to induced fracturing in the caries-resistant inbreds. It was supposed at the outset that if the two strains showed a significant difference in this experiment, a similar difference with respect to fracturing might cause the molar teeth of the susceptible strain to fracture more frequently than in the other strain during mastication, leading to earlier and more frequent caries in the former.

PROCEDURE

A simple apparatus was used to test resistance to breakage in the lower molars. An aluminum rod was dropped vertically through varying distances upon the cusps of molar teeth imbedded in a half jaw which had been dis-

This investigation was supported by a very generous grant from the United States Public Health Service.

Received for publication, July 5, 1951.

*E. C. Nakfoor made all the observations on the fracturing of the molar teeth, and all the quantitative calculations based on these observations.

sected a few minutes previously from an etherized rat. The effect of the impact upon the cusp was observed through a binocular microscope at the instant of impact. The aluminum rod was 7 inches long, cylindrical, $\frac{3}{16}$ of an inch in diameter, and weighed 8.55 grams. The lateral surface of the rod was polished with a fine grade of emery cloth to reduce friction. The rod was coated with a good grade of lubricating oil and then wiped dry before each testing period. Frictional resistance was thereby reduced. The lower end of this aluminum plunger was tapered to a blunt point about one millimeter in diameter.

The aluminum rod was mounted in a supporting apparatus constructed as follows. Two pieces of oak ($\frac{7}{8}$ inch thick and 8 inches wide) were fastened to each other at a right angle. The perpendicular piece was 10 inches high and the base 12 inches long. Attached to the vertical piece of wood was a bar of steel whose two ends were bent accurately at right angles so that they were perpendicular to the vertical support but parallel to the base. Through each end of this bar was drilled a hole, so that there were two bearing surfaces to guide the aluminum rod, with a minimum of friction, when it was dropped upon the cusp of a tooth. A small screw-type vise was imbedded in a metal can filled with concrete. The jaw was held in this vise, which was placed in the line of travel of the aluminum rod. An L-shaped steel bar, attached to the vertical wooden support, regulated the distance through which the aluminum plunger was dropped.

The kinetic energy required to break a cusp could be calculated from the formula, K.E. = M a s, in which M is the mass of the aluminum rod, a the acceleration of a freely falling body, and s the distance the rod was dropped. The same rod was used throughout the experiment, so that M and a were constant factors. The distance, s, through which the rod must be dropped to fracture a cusp was therefore considered a fairly accurate measure, for comparative purposes, of the resistance of teeth to mechanical fracturing.

The animals for this experiment were bred from the caries-susceptible and caries-resistant stocks of Hunt and Hoppert. Nineteen susceptible breeders, comprising 15 crosses, and 21 resistant breeders, making 16 crosses, were taken from Hunt and Hoppert's twentieth susceptible and sixteenth resistant generations, respectively. These breeders produced the young whose teeth were tested. The diet was the same as that devised by Hoppert, Webber, and Canniff[1, 2] except that rice flour was substituted for the more coarsely ground rice to prevent early dental decay.

Ten sites (cusps) on each jaw were chosen for tests on resistance to breakage (Fig. 1). The numbering of the sites began at the anterior median cusp of the first molar and continued posteriorly on the lingual side to the posterior region of the second molar, then proceeded anteriorly on the labial side to the anterior cusp of the first molar. Cusps I, II, III, XII, XIII, and XIV were in the first molar. The second molar contained IV, V, X, and XI. The third molar has not proved important in Hunt and Hoppert's researches, so it was not tested.

Twenty-five of the 58 susceptible rats tested developed caries before the age of 60 days, in spite of the fact that rice flour instead of the coarsely ground rice was used in the ration (Table I). This is contrary to the findings of

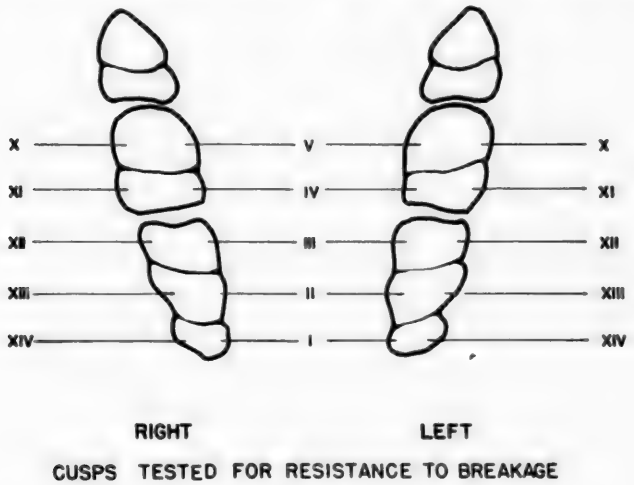

Fig. 1.

Braunschneider, Hunt, and Hoppert.[10] The rice flour used in this experiment was secured from the same firm that supplied Braunschneider's flour, and both flours were processed in a similar manner. The reason for the discrepancy is unknown. There is no evidence of marked increase in susceptibility to caries in the susceptible line since Braunschneider's experiments. The cavities in this investigation were found with the low power of the binocular microscope, while Braunschneider used only the unaided eye. One might think, therefore, that Braunschneider may have overlooked many small cavities, but carious destruction proceeds so rapidly in our susceptible stock that such an explanation seems inadequate. On one occasion coarse rice particles, similar to those found in our regular cariogenic diet, were found in one feed can. We do not know how these particles got there. Two very trustworthy persons, in addition to one of the authors (E.C.N.), occasionally fed the animals. It is possible, but not probable, that the regular caries-producing diet was occasionally, by accident, placed in the feed cans.

Diammonium phosphate (1 per cent by weight) was added to the diet in an attempt to check this unexpected dental caries. Tooth decay in the susceptibles nevertheless continued to appear at about the same rate. This unanticipated dental caries when rice flour was used in the ration merits further investigation.

The lower molar teeth were tested for resistance to fracturing when a rat had become about 60 days old. The ages of the susceptibles tested ranged from 59 to 62 days. The range of the resistants was from 60 to 64 days, except for 4 animals, which were 67 days old. The animal was etherized, the jaw was

TABLE I

AVERAGE DROP DISTANCES (IN MILLIMETERS) REQUIRED TO CHIP OR SPLIT LOWER MOLAR TEETH AT THE SITES INDICATED

	TOTAL NO. OF ANIMALS	I AV. DROP DISTANCE	II AV. DROP DISTANCE	III AV. DROP DISTANCE	IV AV. DROP DISTANCE	V AV. DROP DISTANCE	X AV. DROP DISTANCE	XI AV. DROP DISTANCE	XII AV. DROP DISTANCE	XIII AV. DROP DISTANCE	XIV AV. DROP DISTANCE
RESISTANTS (GROUP R)											
Nonammoniated diet	41	28.2 (17)	21.5 (36)	14.5 (41)	13.9 (40)	13.7 (41)	23.9 (41)	28.0 (40)	28.5 (41)	28.6 (36)	25.9 (23)
Ammoniated diet	16	27.0 (10)	19.3 (15)	13.8 (16)	13.4 (16)	13.8 (16)	20.9 (16)	29.3 (15)	22.8 (16)	25.9 (16)	28.5 (10)
Total	57	27.8 (27)	20.9 (51)	14.3 (57)	13.8 (56)	13.7 (57)	23.1 (57)	28.4 (55)	26.9 (57)	27.8 (52)	26.7 (33)
SUSCEPTIBLES WITH CARIES (GROUP Sc)											
Nonammoniated diet	6	27.5 (2)	20.0 (3)	11.7 (6)	10.0 (6)	7.0 (5)	10.0 (3)	15.8 (6)	20.8 (6)	25.0 (5)	30.0 (3)
Ammoniated diet	19	27.2 (9)	23.5 (10)	12.6 (19)	11.6 (19)	8.7 (19)	9.6 (12)	19.7 (19)	17.1 (19)	24.7 (18)	27.5 (8)
Total	25	27.3 (11)	22.7 (13)	12.4 (25)	11.2 (25)	8.3 (24)	9.8 (15)	18.8 (25)	18.0 (25)	24.8 (23)	28.9 (11)
WITHOUT CARIES (GROUP S)											
Nonammoniated diet	7	26.4 (7)	20.0 (7)	11.4 (7)	13.6 (7)	11.4 (7)	19.3 (7)	20.7 (7)	25.0 (6)	29.2 (6)	21.4 (7)
Ammoniated diet	26	26.8 (19)	23.6 (21)	12.5 (26)	11.9 (26)	11.4 (26)	16.7 (26)	20.8 (24)	21.7 (23)	27.2 (23)	26.7 (18)
Total	33	26.7 (26)	22.7 (28)	12.3 (33)	12.3 (33)	11.1 (33)	17.3 (33)	20.8 (31)	22.4 (29)	27.6 (29)	25.2 (25)
ALL SUSCEPTIBLES COMBINED	58	26.9 (37)	22.7 (41)	12.3 (58)	11.8 (58)	9.9 (57)	14.9 (48)	19.9 (56)	20.4 (54)	24.4 (52)	25.8 (36)

The figures in parentheses are the numbers of cases, which in some instances are less than the total number of animals, because a fracture in one cusp may have injured an adjacent cusp, making it impossible to test the latter.

immediately separated from the head, the flesh removed, the molar teeth scrubbed with a small toothbrush to remove food particles, and the half of the jaw to be tested was placed in the vise and examined with a needle probe under the binocular microscope for caries, natural fractures, etc. The other half of each jaw was labeled with India ink and kept as a control.

About half the jaws tested for resistance to fracturing were right jaws, and the other half left jaws. It was suspected that the molars on the right jaw might differ from those on the left, for Hunt and Hoppert[14] found that the right lower molars of both susceptible and resistant rats showed a greater incidence of caries than the left lower molars.

The teeth were tested in the fracturing machine immediately after they were microscopically examined. Each cusp was subjected to repeated blows by the aluminum rod in the following manner. The point of the rod was aligned with Site I and dropped on it through a distance of 5 mm. The rod was dropped 5 times on this cusp from this height, the cusp was examined with the binocular microscope after each blow, the damage, if any, being recorded after each impact. The cylinder containing the vise was then moved until Site II was in line with the plunger point, whereupon 5 blows from a height of 5 mm. were again administered. This procedure was repeated until every one of the 10 sites had been subjected to 5 blows from a height of 5 mm. The results were continually observed with the binocular microscope. Cusps I, II, III, IV, V, X, XI, XII, XIII, and XIV were tested in that order.

After the drops at the 5 mm. level were completed, the distance was increased to 10 mm., and from this height all sites were again tested. Then tests were made with drops of 15, 20, 25, 30, etc. mm.

OBSERVATIONS

In general, 4 types of damage were observed. Minute particles of tooth substance were knocked off with even the lightest blows; this was called flaking. In cracking, a distinct cleavage was produced, though no part of the tooth was lost. In chipping, actual chunks of the cusp were broken off. Often a whole cusp was broken off; this was defined as splitting. The site was tested until either a large portion of the cusp was chipped off or a whole cusp was split off. When a cusp was chipped or split, the corresponding dropping distance was recorded as the measure of resistance of the cusp to breakage, and it was not tested further.

Table I gives the average drop distances, in millimeters, required to chip or split the lower molar teeth at the sites defined (I, II, III, etc.) in Fig. 1. The data are given for the 57 resistant rats (Group R), for the 25 susceptibles that developed caries (Group Sc), and for the 33 susceptibles that did not show caries (Group S). The observations on animals fed the ammoniated diet and those that were not given it are included.

The resistance to induced fracturing in the two resistant groups was greatest at Site XI, where the average breaking drop distance was 28.4 mm. for 55 animals. This was closely followed by I and XIII (both 27.8 mm.), by XII

(26.9 mm.), and XIV (26.7 mm.). The other average breaking drop distances were at X (23.1 mm.), at II (20.9 mm.), III (14.3 mm.), IV (13.8 mm.), and at V (13.7 mm.).

Unfortunately, the data for the susceptibles with caries (Group Sc) are probably of scant significance in this research, at least for some of the sites, because carious cavities doubtless lowered resistance to induced fracturing.

Upon averaging the observations on the susceptible rats that did not develop caries (Group S), Site XIII showed the greatest resistance to induced fracturing, with an average breaking drop distance of 27.6 mm. for 29 animals. The records for the other sites were: I (26.7 mm.), XIV (25.2 mm.), II (22.7 mm.), XII (22.4 mm.), XI (20.8 mm.), X (17.3 mm.), III and IV (12.3 mm.), and V (11.1 mm.).

Table II shows the differences between the means for the resistants ($\bar{R}$)

TABLE II

DIFFERENCES IN MEAN RESISTANCE TO FRACTURING BETWEEN THE RESISTANT STOCK ($\bar{R}$) AND THE SUSCEPTIBLES THAT DID NOT SHOW DENTAL CARIES ($\bar{S}$)

SITE TESTED	$\bar{R} - \bar{S}$ (MM.)	t VALUE
I	+1.1	0.8
II	−1.8	1.6
III	+2.0	2.7
IV	+1.5	2.0
V	+2.6	4.9
X	+5.8	4.9
XI	+7.6	7.2
XII	+4.5	3.8
XIII	+0.2	0.2
XIV	+1.5	1.2

and the susceptibles without caries ($\bar{S}$). The molars of the susceptibles were more readily broken at all sites except Site II. The mean dropping distances of the aluminum rod were significantly greater in the resistant rats, as judged by the t test, at Sites III, IV, V, X, XI, and XII. The most significant difference was at cusp XI, where the t value was 7.2. Thus it is clear that on the whole it required more energy to break a lower molar tooth in the resistant animals than in the susceptibles. One might be tempted to infer that greater resistance to dental caries is due to greater resistance to natural fracturing. Nevertheless, further observations showed that such a conclusion would be unjustified.

Twenty-five of the 58 susceptible strain rats used in the investigation produced carious cavities in their lower molars. These cavities were identified under a binocular microscope. If natural fracturing is highly important in the origin of cavities, a considerable amount of such fracturing should have been present in the teeth of these animals, for they were obviously at a stage at which caries was being initiated. The control lower half jaws of more than 50 susceptible rats were re-examined with the binocular microscope, and only 3 instances of natural fracturing were found. Two of these were of minor chipping at positions II and IV. The third case was a sharp edged indentation

between cusps V and X. Only one instance of natural fracturing was observed in the resistants, this being a case of minor chipping. Natural fracturing, therefore, did not appear to be of much importance in tooth decay in these animals.

DISCUSSION

Further observation and reflection suggest that the relative weakness of the molars in susceptible rats may have been the result, not the cause, of caries. Staining of the teeth is often an indication of the carious process.[15, 16, 17] The teeth of the susceptible animals showed a greater amount of staining than the resistants. The control half jaws were examined again, this time for staining. Five areas where staining frequently occurs were chosen for study. Among 54 susceptible control half jaws examined, only 4 showed very little or no staining at the selected sites; the remaining 50 showed staining. Two hundred seventy sites were examined; of these, 211 showed discoloration. Even the jaws in Group S showed staining in most instances.

Among the resistant control half jaws, 39 out of 52 jaws examined were negative for staining. Only 24 out of the 260 sites were stained.

A reasonable interpretation of these facts is that in nearly all the susceptible lower molar teeth the carious process had begun, but that in the majority of cases it had not progressed far enough to produce a cavity that could be seen easily with the binocular microscope. This interesting possibility merits further study, particularly because it assumes that caries can develop slowly when the rice of the ration is in the form of flour.

The relative weakness of the molars of susceptibles may also be an indication of a structural difference. An impression which has grown throughout this investigation is that the crevices in the susceptible strain's molars are wider than the crevices in resistant teeth. This impression has been supported by observations both with the unaided eye and with the binocular microscope. This point has not yet been tested with scientific finality, yet it appears to be true. If this view is correct, perhaps another cause for the greater mechanical weakness of the susceptible teeth is that there is not so much material in them.

Wider crevices might, in addition, result in more impaction of food, which in turn might favor acid formation by bacteria, and finally caries. As a matter of fact, 60.4 per cent of the caries in the susceptible line rats has occurred at the major crevices of the first and second lower molars, while in the resistants only 15.7 per cent of the cavities appeared at these locations.[14] Perhaps in our susceptible animals a high incidence of early caries and mechanical weakness of the molar tooth have a common cause—width of the crevice.

CONCLUSIONS

1. The lower molars of resistant 60-day-old rats showed a greater resistance to fracturing when subjected to blows by a tapered aluminum rod than the lower molars of susceptible rats.

2. Unexpected dental caries appeared in some of the susceptible rats fed on the diet containing rice flour, before they were 60 days old.

3. Fracturing was probably not an important factor in initiating dental caries in our rats. Very few fractures were observed in the lower molar teeth of 60-day-old susceptible rats, though dental caries did appear in nearly half of these animals.

REFERENCES

1. Hoppert, C. A., Webber, P. A., and Canniff, T. L.: The Production of Dental Caries in Rats Fed an Adequate Diet, *Science* **74**: 77, 1931.
2. Hoppert, C. A., Webber, P. A., and Canniff, T. L.: The Production of Dental Caries in Rats Fed an Adequate Diet, *J. D. Res.* **12**: 161, 1932.
3. Hunt, H. R., and Hoppert, C. A.: Inheritance in Rat Caries, *Genetics* **24**: 76, 1939.
4. Hunt, H. R., and Hoppert, C. A.: Inheritance in Rat Caries, *Year Book of the American Philosophical Society* **131**: 1941.
5. Hunt, H. R., and Hoppert, C. A.: Inheritance of Susceptibility and Resistance to Caries in Albino Rats (Mus norvegicus), *J. Am. Col. Dentists* **11**: 33, 1944.
6. Hunt, H. R., Hoppert, C. A., and Braunschneider, G. E.: Hereditary and Environmental Factors Influencing Tooth Decay in the Albino Rat (Rattus norvegicus), *Records of the Genetics Society of America* **16**: 37, 1947.
7. Hunt, H. R., Hoppert, C. A., and Erwin, W. G.: Inheritance of Susceptibility to Caries in Albino Rats (Mus norvegicus), *J. D. Res.* **23**: 385, 1944.
8. Hunt, H. R., and Hoppert, C. A.: Occlusion as a Factor in Dental Caries of Albino Rats (Rattus norvegicus), *J. D. Res.* **27**: 553, 1948.
9. Hunt, H. R., and Hoppert, C. A.: Sex and Dental Caries in Albino Rats (Rattus nervegicus), *J. D. Res.* **27**: 486, 1948.
10. Braunschneider, G. E., Hunt, H. R., and Hoppert, C. A.: The Influence of Age in the Development of Dental Caries in the Rat (Rattus norvegicus), *J. D. Res.* **27**: 154, 1948.
11. Robinson, H. B. G.: Editorial, *J. D. Res.* **27**: 545, 1948.
12. Rosebury, T.: Dental Caries, New York, 1941, American Dental Association, p. 200.
13. Rosebury, T.: The Problem of Dental Caries, in Dental Science and Dental Art (S. M. Gordon, editor), Philadelphia, 1938, Lea and Febiger.
14. Hunt, H. R., and Hoppert, C. A.: The Distribution of Carious Cavities in the Lower Molars of Caries-Susceptible and Caries-Resistant Albino Rats (Rattus norvegicus), *J. D. Res* **29**: 157, 1950.
15. Bunting, R. W.: Text-Book of Oral Pathology, Philadelphia, 1929, Lea and Febiger.
16. Hill, T. J.: Text-Book of Oral Pathology, Philadelphia, 1945, Lea and Febiger.
17. Gottlieb, B.: Dental Caries, Philadelphia, 1947, Lea and Febiger.

STUDIES IN THE SYRIAN HAMSTER

II. Blood Sugar; Method and Results

ROBERT E. SULLIVAN, B.A., AND FRANK G. EVERETT, M.S., D.M.D., M.D.

University of Oregon Dental School, Portland, Ore.

THE Syrian hamster (*Cricetus auratus*) has become a commonly used laboratory animal only since 1930.[1] In 1942 Arnold[2] introduced this animal for experimental research in dental caries. The study of this disease in the hamster is particularly relevant because, as in the human dentition, the crowns of the erupting hamster molars are completely covered with enamel.[3] In the course of our own dental caries studies, both past[4] and present, we have been concerned with establishing average normal blood sugar values in this species; in reviewing the literature very little could be found concerning both actual values and methods of drawing blood. Chen, Powell, and Maze[5] gave values in 15 older animals, 10 to 14 weeks old and ranging in weight from 65 to 132 grams. They describe a method using a telescope-shaped holder to restrain the animal, shaving the hind leg and drawing the blood from the superficial vein. This method is rather complicated even in the older animals on which it was used in their laboratory; in freshly weaned animals (30 to 60 grams) it has proved nearly impossible to attain in our hands. Venepuncture in the hamster has been found extremely difficult by Cohen and Fosdick,[6] who found that the animal's blood clotted rapidly when drawn. In view of these difficulties, we believe it worth while to describe the procedure we have used for more than a year for securing the blood, and to present the blood sugar values we have obtained in the Syrian hamster. We have dealt with animals ranging in weight from 21 to 142 grams; our rate of mortality attributable to the procedure of drawing blood has been relatively small (2.0 per cent).

EXPERIMENTAL

The following equipment is used in the procedure: an open glass ether jar 6½ inches wide, 8¼ inches high; 0.1 ml. volumetric pipettes (TC); ¼ c.c. tuberculin syringes with Luer lock; gauge 27, ¾ inch injection needles; paraffin blocks 10 cm. by 5 cm. containing 6 smooth round depressions to receive aspirated blood. Syringes and pipettees are prepared by coating the inside with a fine film of potassium oxalate. For the pipettes this preparation is accomplished by drawing a 2 per cent solution of potassium oxalate through them and allowing them to dry. In the syringes a 2 per cent solution of potassium oxalate is drawn through the barrel and attached needle and expelled immediately before the cardiac puncture.

About 10 c.c. of ether (c. p. for anesthesia) are placed in the ether jar beneath a small towel folded so that with its 4 ends tucked in it fits smoothly into the bottom of the jar. The animal is weighed and dropped into the jar, which is left uncovered so that an adequate amount of oxygen may reach the animal.

This investigation was supported in part by a research grant from the National Institute for Dental Research of the National Institutes of Health, Public Health Service.

Received for publication, Aug. 25, 1951.

Sufficient concentration of ether will be available as the ether vapors are heavier than air. The animal must be observed very closely while anesthesia is obtained. The excitement stage is easily recognized by jumping motions of the animal. Seconds later there is a lack of coordinated movement and the animal will fall. It may then be placed on its back, but is still left in the jar. Respiratory movements will then become jerky and the animal may cease to breathe for 2 or 3 seconds. Respiration then becomes regular again, indicating entry into the first phase of the surgical stage. No running movement is present at this time. Now the animal is removed from the jar and placed on its back with its hind legs pointing toward the worker, who places his left index finger very lightly on the left chest in the precordial region to detect the apical impulse. The needle is inserted frontally from caudally and the animal's left toward dorsal, cranial and right in a very steep grade, nearly at right angles to the skin surface. The left hand now grasps the barrel of the syringe and the right hand pulls the plunger back slightly. If there is difficulty in drawing blood, it is suggested that the thumb and index finger of the left hand only be used to hold the barrel, and that the middle finger of that hand be used to hold the heart in place lightly by placing that finger to the right of the midline in the region of the heart. This suggestion is made because the heart of the hamster is easily moved by the tip of the needle. The needle should not be inserted too deeply, only 3 or 4 mm. under the skin. If further difficulty is encountered in drawing the blood, the needle should be withdrawn about 1 mm. and the plunger pulled back again. Twenty one-hundredths of a cubic centimeter of blood are obtained from intracardially and immediately expelled into one of the excavations of the paraffin block. The amount of blood drawn should be about double the amount needed, to minimize the inaccuracy involved by oxalating the syringe. With the pipette, 0.1 mm. of blood is taken up and immediately expelled into a centrifuge tube containing 7.5 mm. of distilled water. From here on Somogyi's modification[7] of the micro method of Shaffer-Hartmann is used. In about 1 case in 10 we have drawn arterial blood; in the majority of casees we have drawn venous blood. It is understood that this may make some difference in the values obtained[8] but no effort has been made to establish separate values for either type of blood in the heart chambers.

RESULTS

Using the procedure described, we determined blood sugar values for normal male Syrian hamsters on each of 2 diets, described in Table I. Blood sugar values were established for animals 28 to 40 days old, weighing from 30 to 66 grams, and also for animals 80 to 120 days old, weighing from 78 to 142 grams. They were not starved prior to the drawing of blood because a long period of starvation is necessary to obtain fasting blood sugar values, due to storage of food in the animals' pouches. The blood sugar values established by using Somogyi's modification of the micro method of Shaffer-Hartmann[7] are shown in Table I. They are in rather close agreement with Chen, Powell and Maze[5] who found the value 92.5 ± 4.1 mg. per 100 c.c. in their 15 older animals, using the Hagedorn-Jensen method.[9]

SUMMARY

A method of heart puncture for the drawing of blood in the Syrian hamster is described. In the use of this procedure a very low mortality rate has been obtained. The actual time expended per animal, including weighing and anesthesia, is not more than 3 or 4 minutes. Hamster blood, previously reported as clotting very rapidly,[6] is handled without such difficulty. Blood sugar values are given for young and adult animals on synthetic high sucrose, and on natural maintenance diets.

TABLE I

BLOOD SUGAR VALUES IN THE SYRIAN HAMSTER

DIET*	NUMBER OF ANIMALS	AGE (DAYS)	BLOOD SUGAR MG./100 C.C.
1	26	28-30	92.3 ± 2.8
1	24	80-120	88.9 ± 3.7
2	92	30-40	92.9 ± 2.8
2	19	80-120	97.3 ± 7.1

*Diet No. 1 consisted of Purina Laboratory Chow and water, both ad libitum, with semi-weekly supplements of raw liver and green and yellow vegetables. Diet No. 2 was the one described by Shaw, Schweigert, McIntire, Elvehjem and Phillips,[10] and modified by Shaw.[11] This diet consists of sucrose 67 parts, casein 24 parts, salts 4 parts and corn oil 5 parts. To this are added the vitamins, liver 2 parts and liver concentrate 2 parts.

REFERENCES

1. Adler, S.: Origin of the Golden Hamster Cricetus auratus as a Laboratory Animal, *Nature* **162**: 256, 1948.
2. Arnold, F. A., Jr.: The Production of Carious Lesions in the Molar Teeth of Hamsters (C. auratus), *Public Health Rep.* **57**: 1599, 1942.
3. Keyes, P. H., and Dale, P. P.: The Preliminary Survey of the Pouches and Dentition of the Syrian Hamster, *J. D. Res.* **23**: 427, 1944.
4. Everett, F. G., Lal, D., and Phatak, N. M.: The Effects of Alloxan Diabetes on the Incidence of Dental Caries in the Syrian Hamster (Preliminary Report), *J. D. Res.* **28**: 653, 1948.
5. Chen, K. K., Powell, C. E., and Maze, N.: The Response of Hamsters to Drugs, *J. Pharmacol. & Exper. Therap.* **85**: 348, 1945.
6. Cohen, B., and Fosdick, L. S.: Chemical Studies in Periodontal Disease. VI. The Glycogen Content of Gingival Tissue in Alloxan Diabetes, *J. D. Res.* **29**: 48, 1950.
7. Shaffer, P. A., and Somogyi, M.: Copper-Iodometric Reagents for Sugar Determination, *J. Biol. Chem.* **100**: 695, 1933.
8. Chauvetau, M.: Nouvelles recherches sur la question glycogenique, *Comptes rendus des Seances de l'Academie des Sciences* **42**: 1008, 1856.
9. Hagedorn, H. C., and Jensen, B. N.: Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid, *Biochem. Ztschr.* **135**: 46, 1923.
10. Shaw, J. H., Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Phillips, P. H.: Dental Caries in the Cotton Rat. I. Methods of Study and Preliminary Nutritional Experiments, *J. Nutrition* **28**: 333, 1944.
11. Shaw, J. H.: Carious Lesions in Cotton Rat Molars. I. The Role of Mechanical Factors Studied by the Extraction of Antagonistic Molars, *J. D. Res.* **26**: 47, 1947.

Erratum

On page 824 of the December issue of the JOURNAL, in the article entitled "Dolorimetric Evaluation of Dental Patients" by Austin H. Kutscher and Neal W. Chilton, the expression in Table II reads:

"*Standard deviation $\sigma = \sqrt{\frac{\Sigma x^2}{N}} - x^{-2} \ldots$"

The last part of this expression (x^{-2}) should read $\bar{x}^2$.